RIJKSUNIVERSITEIT GRONINGEN

Seasonal synchronization between trophic levels under climate change
Genetic and environmental effects on winter moth egg hatching

Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
vrijdag 15 juni 2007
om 14.45 uur

door

Margriet van Asch
geboren op 3 juli 1979
te Tiel

Promotor:	Prof. Dr. M.E. Visser
Beoordelingscommissie:	Prof. Dr. J. Ellers
	Prof. Dr. M. Merrow
	Prof. Dr. C. Parmesan

The research presented in this thesis was carried out at the Netherlands Institute of Ecology (NIOO-KNAW), Centre for Terrestrial Ecology, P.O. Box 40, 6666 ZG Heteren, the Netherlands.
The Life Sciences Foundation (NWO-ALW grant 812.04.009) supported this research.

Contents

Chapter 1: General introduction

Introduction

The temperate region is characterized by strong seasonality; hence there is only a limited period each year in which conditions for growth and reproduction are optimal. This optimal moment is determined by both abiotic and biotic factors. Temperature and precipitation are the main abiotic factors. For instance, many deciduous tree species only start developing their leaves in spring, when the temperature starts to increase. Would they start developing earlier, they would risk freezing, as the temperature may drop below 0 °C. The presence (or absence) of other species within an ecosystem are potentially important biotic factors. Specifically the underlying trophic level may be important. A predator can only survive if its prey is present, herbivores need their host plant to feed on and parasites need a host in order to survive. Especially in those cases were there is only a very short time window where the conditions are suitable for growth or reproduction, the question arises how an organism can 'time' its life cycle so as to appear in the right part of the life cycle at the right moment in time.

Just as with many other traits, the timing of life cycle events is affected by both the environment and by the genetic make-up of the individual. Many plants and animals show a plastic response to environmental cues such as temperature. In warm springs insects emerge earlier, birds start earlier with egg laying and trees start leafing earlier. They are so-called phenotypically plastic: the same genotype produces a different phenotype (i.e., phenology) under different environmental conditions (Pigliucci 2001). An individual may be affected directly by its own environmental conditions, or, alternatively by the environmental conditions of its parents. Thus, maternal (or paternal) effects are a special form of environmental effects acting across generations (Mousseau and Dingle 1991) and can thus also potentially affect timing of life cycle events.

Many traits are also genetically determined, and are thus heritable. In general, natural selection is thought to maintain an organisms' optimal timing, as emergence outside the optimal period often has severe fitness consequences, and most traits show a heritable component. Especially in natural populations, environmental and genetic effects are often considered separately. Recently, the focus is shifting towards a reaction norm approach, integrating both environmental and genetic effects (Postma and van Noordwijk 2005; Nussey *et al.* 2007). Recent advances in both molecular techniques and computational methods (e.g., animal models) have facilitated this.

A reaction norm gives the expression of the same genotype along an environmental axis (Scheiner 1993). Individuals may differ both in slope and in intercept of their reaction norm. Variation in intercept of the reaction norm means that individuals differ in mean trait, e.g., some families will always emerge earlier than others, independent of the environmental conditions. On the other hand, some individuals may be more phenotypically plastic than others (variation in slope of the reaction norm). When studying seasonal timing it is important to approach this from a reaction norm perspective, because the environment – and thus the optimal timing for emergence - differs among years. Whether or not a

species can realize an optimal timing in these different years depends on the shape of the reaction norm. Directional selection under one set of environmental conditions affects the response under another set of environmental conditions. Another aspect important when studying seasonal timing, is that of which cues are the relevant ones to determine the moment of emergence (i.e., what to put on the environmental axis of the reaction norm). When studying synchrony between different trophic levels, such as a herbivore and its host plant, it is crucial to know which cues they are both using, as that determines to a large extent under which conditions synchrony can be maintained.

Understanding how organisms can maintain their seasonality is even more crucial in the recent light of climate change. The different trophic levels within a food chain can all be affected by changes in, for instance, temperature. However, there is no *a priori* reason why the different components within the food chain should be affected to the same extend (Stenseth and Mysterud 2002; Visser and Both 2005). A temperature increase may lead to an earlier start of plant development of, for instance, a week. However, this means that the herbivore feeding on that plant species should also advance its timing with a week. Should the same temperature increase lead to an advance of two weeks in the herbivore, this means that there will be no food available to the herbivore during the first week of its development. This could happen if the herbivore and its host use different cues to determine their phenology. If the range of environmental conditions changes, or if the relation between different environmental cues changes, then the outcome of the mechanisms determining herbivore and plant phenology may suddenly be different. Therefore understanding how the timing of the different life stages is determined is also crucial when predicting the effects climate change will have on ecosystems.

Study system

I use the winter moth (*Operophtera brumata*) feeding on oak (*Quercus robur*) as a model system. Winter moths have an annual life cycle. Larvae hatch in early spring, and they feed on the young leaves from deciduous tree species. Within the Netherlands they are a common species of insect herbivore, feeding predominantly on oak. After four to six weeks the larvae descend from the canopy of the tree, and pupate in the soil. There they remain until late autumn/early winter, when the adults emerge from the pupae. Male winter moths can fly, but the females are wingless. They climb the trunk of the tree, and they lay their eggs in the upper branches. There the eggs remain until egg hatch the following spring.

Timing of egg hatch in spring is crucial to the winter moths, as there is only a short time window in which the oak leaves are suitable as a food source to the larvae. To obtain the highest pupation weight, and thus fecundity, egg hatching should coincide with oak bud opening. Early hatching results in high mortality due to starvation, while late hatching leads to a reduction in fecundity. Oak bud opening varies between years, and thus the optimal

moment of egg hatching also varies over the years. Therefore this is a study system well suited to study the factors determining egg hatch, and thus synchrony with the host plant.
At the Netherlands Institute of Ecology (NIOO-KNAW) in Heteren winter moths have been studied since 1994. Each autumn traps are put up on trees, each year on the same trees in the same forests. These are then checked two to three times a week, and the adult moths are collected. Each year eggs from female moths are then kept in an insectarium in Heteren under 'outdoor' conditions, and in spring egg hatching is monitored. Development of the oaks is also determined during regular (2-3 times per week) visits to each forest.
Winter moths were continuously studied during the whole period in three Dutch forests: Oosterhout (51°55' N, 05°50' E), Doorwerth (51°59' N, 05°48' E) and Warnsborn (52°05' N, 05°50' E). In addition I collected data in three other forests in the period 2003-2005 (Hoge Veluwe (52°02' N, 05°51' E), Rhenen (51°57' N, 05°35' E) and Wolfheze (51°59' N, 05°47' E).

Outline of the thesis

In this thesis I start by reviewing the relevant literature on synchrony between forest caterpillars and their host plants. I do this in chapter 2, looking at factors at different levels affecting synchrony. I try to link mechanisms, adaptation, and population dynamics all within a single framework, that is needed for a full understanding of the causes and consequences of the (a)synchrony.

To understand the proximate factors affecting synchrony, we need to look at the mechanisms determining both insect and host plant phenology. We need to understand how both the insect and the host plants respond to different environmental conditions. Although descriptive (correlative) models of both oak bud opening and winter moth egg hatching exist, we have some indication that - at least for the moths - these do not necessarily reflect the causal mechanisms. In chapter 3 we explore into more detail the mechanism determining winter moth egg hatch, determining the exact (nonlinear) response of the eggs to temperature, and looking at changes in temperature sensitivity over time. Egg hatching may also be affected by environmental factors other than temperature. In chapter 3 we also check whether eggs are photoperiod sensitive. Environmental effects can act across generations, and thus parental effects can potentially affect timing offspring emergence. In chapter 4 we therefore study the effect of maternal feeding conditions on egg hatching date in the offspring.

In order to adapt to host plant phenology, sufficient genetic variation is needed as well as selection pressures. In chapter 5 we look at the genetic variation in egg hatching reaction norm (i.e., the phenotypic response to different temperatures for different genotypes). We then combine this with the experimentally determined selection pressures and a climate scenario in order to predict (genetic) changes in winter moth egg hatching under climate change. In chapter 6 we compare the predicted changes in egg hatching phenology with the actual, observed changes. We do this both by looking at long term (1995-2006) common

garden data collected in Heteren on winter moth egg hatching, and also by experimentally comparing reaction norms in two years (2000 and 2005).

Finally, I will give a summary and discuss the results in chapter 7.

Chapter 1

Chapter 2: Phenology of forest caterpillars and their host trees: the importance of synchrony

Margriet van Asch and Marcel E. Visser

Netherlands Institute of Ecology (NIOO-KNAW), 6666 ZG Heteren, The Netherlands; email: M.vanAsch@nioo.knaw.nl, M.Visser@nioo.knaw.nl

Published in Annual Review of Entomology 52: 37-55 (2007)

Abstract

For many leaf-feeding herbivores, synchrony in phenology with their host plant is crucial as development outside a narrow phenological time window has severe fitness consequences. In this review, we link mechanisms, adaptation, and population dynamics within a single conceptual framework, needed for a full understanding of the causes and consequences of this synchrony. The physiological mechanisms underlying herbivore and plant phenology are affected by environmental cues, such as photoperiod and temperature, although not necessarily in the same way. That these different mechanisms lead to synchrony, even if there is spatial and temporal variation in plant phenology, is a result of the strong natural selection acting on the mechanism underlying herbivore phenology. Synchrony has a major impact on the population densities of leaf-feeding Lepidoptera, and years with a high synchrony may lead to outbreaks. Global climate change leads to a disruption of the synchrony between herbivores and their host plants, which may have major impacts for population viability if natural selection is insufficient to restore synchrony.

Introduction and conceptual framework

The temperate region is characterized by strong seasonality; hence there is only a limited period each year in which conditions for growth and reproduction are optimal. For many plant herbivores, the phenology of their host plant species determines this period, and it is therefore crucial for these species to be synchronized with their host plant's phenology. Development outside the period of optimal conditions often has severe fitness consequences in terms of reduced survival or fecundity. For leaf-feeding moths (e.g. Feeny 1970; Stoyenoff et al. 1994; Ivashov et al. 2002; Klemola et al. 2003) and aphids (e.g. Akimoto 1998; Ozaki 1998), the most-favorable period is just after the buds of their host plant open because young leaves provide the best food for them. For other species, such as flower- and seed-eating weevils (e.g. Connett et al. 2001; Russell and Louda 2004), the optimal period depends on the flowering date or the period of seed setting of their host plant.

The degree of (a)synchrony between herbivores and their hosts depends by definition on the phenology of both the herbivore and its host. The degree of (a)synchrony is calculated as the difference between the phenological stadia of herbivore and host that are most relevant to the herbivore. Many leaf-feeding moths have to start feeding just after the leaves of their host plant become available. In that case, the time-difference between hatching of caterpillars and the opening of the buds of the host plant is the degree of (a)synchrony, with perfect synchrony when both events happen simultaneously. To quantify these phenological events, measures such as the date of first leaf unfolding and first egg hatch date of the herbivore are used.

To study synchrony, we have to understand the underlying mechanisms determining the phenology of both herbivores and their host plants. The phenology of plants varies from year to year, depending on environmental conditions. Hence, the timing of the optimal period for the herbivore also varies annually. To maintain synchrony with the host plants in different years, many animals also have a plastic response to environmental cues such as temperature. They are so-called phenotypically plastic: The same genotype produces a different phenotype (i.e., phenology) under different environmental conditions (Pigliucci 2001). We may expect that through natural selection, the outcome of the mechanism underlying the herbivore's phenology should match closely the outcome of the mechanism underlying the phenology of its host plant. However, even though the outcome may be similar under some circumstances, the underlying mechanism (i.e., the cues and in the way they are used) may be different for herbivores and their host plants. This may become apparent in an environment that is different from the environment in which the plants and insects have evolved. If the range of environmental conditions changes, or if the relation between different environmental cues changes, then the outcome of the mechanisms determining herbivore and plant phenology may be different. Hence species introductions in a new environment or a rapid change of the environment (for instance, owing to climate change) may lead to a disruption of synchrony. This occurrence leads to directional

selection on the mechanism underlying the herbivore's phenology. If and how fast the herbivores adapt to their new environment then depends on the heritability and the strength of selection. If the response to selection is not strong enough to restore synchrony, this has consequences at the population dynamics level because synchrony is likely linked with population growth of the herbivore.

Although there are many different cases in which synchronization between different trophic levels plays an important role (Visser and Both 2005), in this review we focus mainly on Lepidopteran larvae that feed on the young leaves of deciduous trees because this group is relatively widely studied. Moreover, we further limit our review by looking at synchrony from the viewpoint of the herbivores. Opposing selection pressures exist for herbivore and host plant, since host plants benefit from being asynchroneous with their herbivores. However, the deciduous tree species that serve as host to many of the insect herbivores discussed here have a generation time that is many times that of the herbivores. In general, we expect that most herbivores can more easily keep up with any changes in host phenology than that their hosts can change their phenology to prevent damage. Even though many processes also affect the plant, we only discuss these insofar as they have an effect on host plant phenology.

We address the synchronization of plant and herbivore phenology, linking different levels of biological organization, mechanisms, adaptation, and population dynamics within a single framework (Figure 1). In the first section, we review the mechanisms underlying both insect and host plant phenology. The second section deals with the selection pressures acting on these mechanisms, as well as the possibilities of a genetic response to selection. In the third section, we discuss the impact of synchronization on the population dynamics of insect herbivores, focusing on species with strongly fluctuating population densities. In the fourth section, we discuss how synchrony may be affected by changes in the environment. In the last section, we discuss the focus for future research.

Mechanisms underlying synchrony

The degree of synchrony between herbivore and plant phenology is the result of two underlying processes: the response mechanism of the plant and the response mechanism of the herbivore. We therefore first review the literature on plant and herbivore phenology separately before looking at the combined effect of these on the degree of synchrony.

In general, organisms use cues that correlate with future conditions to predict these conditions. The two most-important cues are temperature and photoperiod. Temperature effects can be divided into two classes that directly relate to the different phases of development. Physiological processes are generally temperature dependent, and thus the developmental rate is higher at a higher temperature. In warmer springs, caterpillars emerge earlier (e.g. Embree 1970; Lysyk 1989; Buse and Good 1996; Andresen et al. 2001), and trees start leafing earlier (e.g. Kramer 1995; Menzel 2000; Root et al. 2003). Both insect and plant species may also have a rest phase with no or little development. This rest phase, termed diapause in insects (Tauber and Tauber 1981), is important because it prevents

development in, for instance, late autumn when conditions can be mild. It also increases resistance to adverse environmental conditions. Environmental cues, mainly photoperiod and temperature, determine both the start and the end of this rest phase.

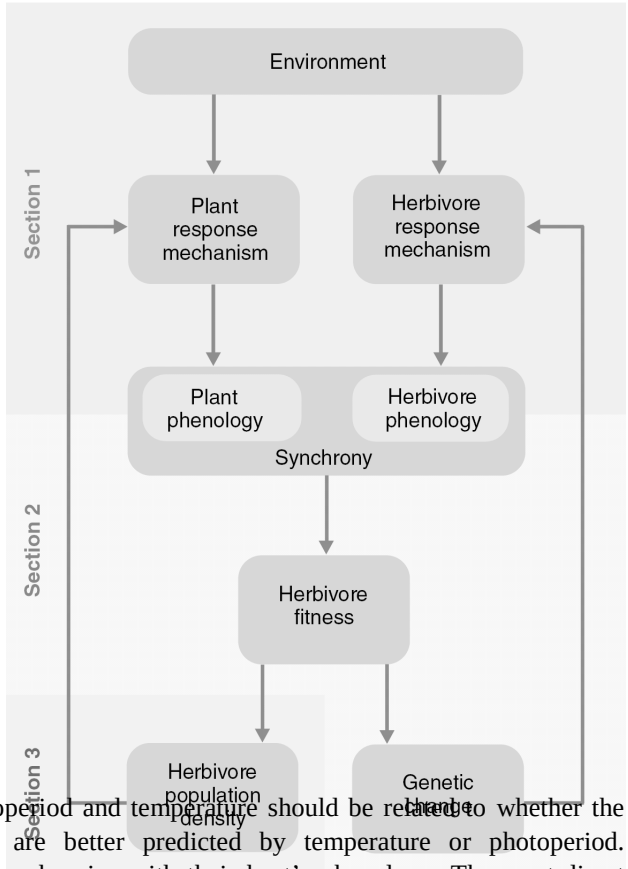


Figure 1 Schematic overview of the topics affecting synchrony and affected by synchrony discussed in this review (see text). The phenology of both the herbivore and the plant depends on their underlying physiology and the environmental cues (first section). Fitness depends strongly on the synchrony of the herbivore's phenology with the phenology of the host plant, and adaptation to the host plant's phenology depends on selection together with genetic variation (second section). Synchrony also affects the population dynamics of the herbivore (third section). Colored shadings accentuate the different sections as discussed in text. Not only are insects affected by synchrony, plants are also affected, but we do not take these into consideration in this review.

The relative importance of photoperiod and temperature should be related to whether the conditions during development are better predicted by temperature or photoperiod. Herbivores use cues mainly to synchronize with their host's phenology. The most-direct cue is a chemical directly associated with bud burst. However, there is no evidence that eggs actually use bud burst as a cue (e.g. Buse and Good 1996). Perhaps egg development has to be initiated much earlier than the moment of bud burst, or perhaps there are simply no detectable cues directly linked to bud burst that the insects can use. Trees may use cues to avoid development when there is still a high probability of frost, which may have dramatic effects on trees when they are just opening their buds (e.g. Hanninen 1991; e.g. Kramer 1994).

Plant Phenology: Bud Opening of Deciduous Trees

In winter, tree buds are still largely undeveloped and in rest, no growth takes place owing to unfavorable physiological conditions within the buds themselves (Sarvas 1974; Kramer

1994). Changing from the rest phase to developmental phase involves mostly a period of chilling (a certain period below a certain temperature): This occurs in species such as *Picea sitchensis* (Cannell and Smith 1983; Cannell and Smith 1986; Murray et al. 1989), fruit trees such as *Malus pumila* (Cannell and Smith 1986), *Fagus sylvatica* (Murray et al. 1989; Kramer 1994), *Betula pendula* (Murray et al. 1989), and *Salix viminalis* (Murray et al. 1989). Chilling usually involves a longer period at approximately 5-7º C (Powell 1987), but in some cases, short-term freezing also releases bud dormancy (Rinne et al. 1997). The effect of chilling can vary between species: Late-developing tree species are much more affected by it than early developing species (Murray et al. 1989). After the buds have experienced a sufficient amount of chilling, they change from the rest phase to the developmental phase. In this phase, the buds start developing, which is only limited by unfavorable environmental conditions (e.g., temperature) (Kramer 1994). If buds are in the developmental phase, but the conditions are such that no development can take place, this is sometimes called the quiescence phase. Development in the active phase is temperature dependent; at a higher temperature, development in the buds occurs faster. The buds open after they have experienced a certain amount of warmth. Thus, temperature clearly affects tree phenology.

Photoperiod can also have an effect on tree phenology (e.g. Hakkinen et al. 1998). Development starts eventually, even if enough chilling has not taken place; photoperiod becomes an overriding cue. Although temperature is highly variable, photoperiod is highly consistent between years. Early developing trees have a higher risk of frost damage. Temperature is a good predictor of this risk. Late-developing species have a more reduced risk, and the moment the buds start developing is more constant between years: They use photoperiod as a cue. Indeed photoperiod affects late-developing species more strongly than early developing trees (Schaber and Badeck 2003).

Insect Phenology: Larval Emergence of Insects

Many insects experience a diapause during winter, e.g., gypsy moths (*Lymantria dispar*) (Gray et al. 1995). Diapause is a hormonally induced state of low metabolic activity, thereby increasing the resistance to unfavorable environmental conditions (Tauber and Tauber 1981). Environmental cues are used to determine both the onset and termination of diapause. Photoperiod is the most-common cue used (Tauber and Tauber 1981; Bale et al. 2002; Denlinger 2002), although several other factors, including temperature, can also affect diapause (Tauber and Tauber 1981). After diapause ends, development can continue; this process is temperature dependent. Thus in those species that have a diapause, photoperiod and temperature act together to determine phenology.

In those species without a true diapause, development is usually also low during winter. This can, however, be fully explained by a low temperature, rather than a change in the metabolic rate within the individual itself. Phenology is usually described using some kind of degree-day model, similar to the models used to describe plant phenology in the developmental phase. These models generally work with a temperature above a certain

threshold, below which it is assumed no development can take place. One can then calculate the temperature above the threshold value and multiply this with the duration of the period (such as days or hours) during which this temperature was experienced. Once a certain value in degree-days has been reached, egg hatch takes place. For example, a species' eggs hatch at 200 degree-days and it only develops above 5 °C. This means that eggs hatch after 40 days at 10 °C (5 degree-days per day), or after only 20 days at 15 °C (10 degree-days per day). Models using degree-days are commonly used on insects, including many Lepidoptera such as Geometridae *(e.g., Operophtera brumata*, Embree 1970; Watt and McFarlane 1991; Dewar and Watt 1992; Buse and Good 1996), Tortricidae (Lysyk 1989), spruce bud worm (*Choristoneura fumiferana*), gypsy moth (*L. dispar*) (Andresen et al. 2001), Cochilidae (Barker and Enz 1993), Carposinidae (Kim et al. 2000; Kim et al. 2001), Pyralidae (Stevenson et al. 2005) and Plutellidae (Kumral et al. 2005), as well as Coleoptera (Grafton-Cardwell et al. 2005), Thysanoptera (Bergant et al. 2005), and Hemiptera (Hill and Hodkinson 1995).

However, even if a model gives a good general description, it does not necessarily reflect the underlying mechanism. Degree-day models assume a linear relationship between temperature and developmental rate. In the intermediate temperature ranges, this relationship is indeed more or less linear, which explains why this kind of model gives a good description. But even in studies where degree-day models give a good description, this relationship is not linear for the more-extreme temperatures (Judd et al. 1991; Barker and Enz 1993): The curve levels off at both the upper and lower temperature range. Owing to enzyme inactivation at these temperatures, physiological processes slow down. Because degree-day models assume a linear relationship between development rate and temperature, these models overestimate development rate at a high temperature. Thus, development takes longer at a high temperature than predicted by a degree-day model. More physiologically based models (Logan et al. 1976; Sharpe and DeMichele 1977; Schoolfield et al. 1981; Lactin et al. 1995) do take this into account, and these models are also known as rate-summation models. Rate-summation models assume a non-linear relationship between development rate and temperature, and they estimate the amount of development during a certain period (e.g., day, hour), given the temperature in that period. The total development then follows from summing of the separate amounts of development, hence their name. These models are traditionally more used in physiological studies than in ecological studies. Rate-summation models actually gave a better fit than degree-day models in a wide range of species, including the adult emergence of several Lepidoptera (e.g., Got et al. 1996; Bryant et al. 2002); although for one species, a degree-day model gave a better fit (Bryant et al. 2002).

Winter moths (*O. brumata*) are another example that show the importance of the nonlinear relationship between temperature and development rate. Although some evidence suggests that an egg diapause exists in some populations, central European winter moth populations do not have an egg diapause (e.g., Embree 1970; Holliday 1985, and references therein). However, a chilling period has been reported to reduce the thermal requirements

(Bonnemaison 1971; Kimberling and Miller 1988). Models including a chilling effect correlate well with egg-hatch phenology (Visser and Holleman 2001). In these models, no development is assumed to take place below a certain threshold temperature, but experiencing temperatures below a threshold value reduced the thermal requirements afterwards. However, if some development can take place while experiencing temperatures below this threshold value, even if only slowly, then slightly less development takes place after the chilling period. Thus egg hatch takes place sooner in the eggs that experienced the chilling period than in those that did not. Indeed, nonlinearity can fully explain differences in the developmental rate between constant and fluctuating temperatures (Bryant et al. 1999). Temperature is thus the main factor affecting herbivore phenology, with photoperiod playing a role in species that have a diapause.

Synchrony in Herbivore and Plant Phenology

The degree of (a)synchrony depends on the difference between the phenology of the host plant and the phenology of the insect herbivore. Temperature affects both, but herbivores and plants may use temperatures from different periods (e.g., early versus late spring temperatures, Visser and Holleman 2001) and hence respond differently to the same increase in temperature. Models used to describe plant and insect phenology come from different fields and are therefore traditionally different in structure. Whether this reflects a difference in the underlying mechanisms is important when assessing how environmental conditions affect synchrony. Unfortunately, few researchers have attempted to model both insect and tree phenology in a single model structure. However, insects and trees likely use different components of their environment. Indeed, as discussed above, trees first break their dormancy before the buds can start developing, whereas nondiapauzing insect herbivores can probably start developing immediately, but low temperatures prevent them from doing so. Winter-diapauzing herbivores first have to finish diapause before they can start developing. Development in winter-diapauzing insects may thus come closer to the processes experienced by the trees than non-diapauzing insects. It would be interesting to test whether insects use the same cues as their hosts, i.e., whether insects feeding on photoperiod-sensitive plants are also sensitive to photoperiod and insects feeding on trees using chilling also use chilling. For instance, *O. brumata* egg hatch depends solely on temperature, and not on photoperiod (Topp and Kirsten 1991 and M. van Asch, unpublished data), as do their host trees, *Q. robur* (Kramer 1994). There is, however, too little data on herbivores and the associated trees to carry out such a test. Moreover, this testing should be done within localities, as adaptation to different environmental conditions may lead to differences in, for instance, bud-dormancy release between populations of the same species (e.g. Li et al. 2004).

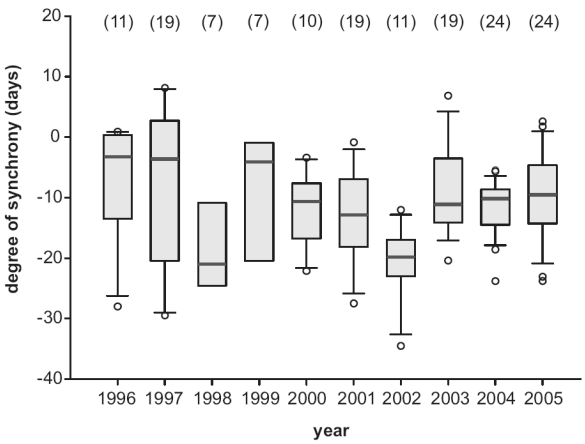


Figure 2 The degree of synchrony with its host plant varies among years and among trees within years for an *O. brumata* population living on *Q. robur*

in the Netherlands (M. van Asch & M. E. Visser, unpubl. data). Degree of synchrony is given by the difference (in days) between the median egg hatch date and the median bud opening date of the crown of the specific host tree of the eggs. Numbers between brackets represent number of trees within each year sample; negative y-values denote egg hatching before bud opening.

The degree of synchrony can vary strongly between years (Figure 2). This variation provides direct evidence that the underlying mechanisms respond differently to environmental conditions, even though the mechanism determining insect herbivore phenology is selected to mimic as closely as possible the response of the host plant. Herbivores and plants use different components of their environment; thus the correlations between these components are of crucial importance: If chilling in early winter is important for the trees, whereas the spring temperatures matter more for the insects, the phenology between herbivore and host is only synchronized in years when the winter and spring temperatures are well correlated. High spring temperatures after a cold winter may lead to asynchrony. The more different the mechanisms underlying the phenology of the plants and the herbivores are, the more critical these correlations between the environmental components are.

Evolutionary ecology

Fitness Consequences of Synchrony

The degree of synchrony has consequences for the fitness of an insect herbivore. If the phenology of the insect does not match the phenology of the host plant, the severity of the fitness consequences depends on the width of the time window in which leaves, seeds, or flowers are suitable for consumption to the insect herbivore, but also on, for instance, the starvation tolerance of the herbivore.

Many leaf-feeding Lepidoptera depend on young, newly grown foliage in spring. If eggs hatch before the tree buds start opening, the larvae of most species can survive for a couple of days without food, but after that, mortality rate increases fast. Four to five days of starvation are sufficient to kill more than half of winter moth larvae (Wint 1983; Tikkanen and Julkunen-Tiitto 2003), and the highest mortality of winter moths “occurs in the first larval stage” (Varley and Gradwell 1960). Gypsy moths (*L. dispar*) can survive longer without food than winter moths (Hunter 1993), although survival also depends on the weather during the starvation period. *Tortrix viridana* can survive starvation relatively well; for example, 80% of the larvae can survive 10 days of starvation (Hunter 1990).

In the absence of food, larvae may disperse to nearby host plants. They can do this by a process called ballooning: Larvae spin a silk thread and then let themselves be blown away by the wind. Estimates of dispersal distances range from only a couple of meters (e.g. (e.g., Harrison 1994) to several 100 meters (e.g., Diss et al. 1996, and references therein), depending on the species. Studies measuring survival probability during and after ballooning have been rare, but mortality is assumed generally to be high and to increase with increasing dispersal distance (Zalucki et al. 2002). Hatching before bud burst thus has severe costs. Conversely, hatching too late is also unfavorable. One of the mechanisms that prevents a plant from being eaten is the use of defensive compounds. In maturing leaves of deciduous trees, leaf toughness (e.g., Feeny 1970) (e.g., 23) and the concentration of defensive compounds like tannins increases (Feeny 1970; Tikkanen and Julkunen-Tiitto 2003), whereas nitrogen and water content decreases (Feeny 1970); the leaves become increasingly inedible. This may lead to a reduction in survival, growth rate, pupation weight, and fecundity in the insect herbivore. Survival of first instar *Zieraphera canadensis* (Lepidoptera: Tortricidae) feeding on white spruce bud (*P. glauca*) decreases when feeding on buds that have only been opened for three days (Quiring 1992). Moreover, older larvae disperse to parts of the tree with younger buds that only opened after they hatched, rather than remain where they were feeding as first instar (Carroll and Quiring 1994). Many species, including common generalist species like *O. brumata* (Feeny 1970; Van Dongen et al. 1997; Tikkanen and Julkunen-Tiitto 2003) and *Epirrita autumnata* (Haukioja et al. 2002), feeding on older leaves grow slower, are much lighter at pupation and thus have a much-reduced fecundity. Fecundity also depends on the host plant: Winter moths feeding on heather (*Calluna vulgaris*) do not suffer a decline in fecundity when they are fed on much-older heather (Kerslake and Hartley 1997).

The severe fitness consequences of mistiming for insect herbivores can be expressed in a fitness curve (Figure 3). This curve shows a clear maximum for hatching at the time of bud opening. Hatching early leads to increased mortality, whereas hatching later means that the quality of the host plant, and thereby fecundity of the herbivore, is decreased. The exact shape of the fitness curve varies among species, depending on their ability to survive a period without food. For instance, the specialist feeder *T. viridana* (Hunter 1990; Ivashov et al. 2002) has a higher resistance to starvation than the generalist feeder *O. brumata* (Hunter 1990; Tikkanen and Julkunen-Tiitto 2003). The fitness curve also depends on the host plant,

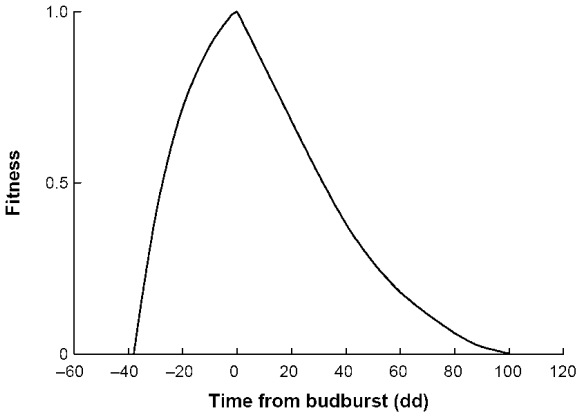


Figure 3 Winter moth fitness depends on degree of synchrony with oak bud opening (Tikkanen and Julkunen-Tiitto 2003). Fitness is expressed relative to the fitness of larvae hatched at bud opening (i.e., in synchrony, dd=0). Degree of synchrony is expressed as

time from bud opening, in degree-days (above 5 ºC); e.g., 1 day at 10 ºC equals 5 degree-days (dd), negative values denote egg hatching before bud opening.

especially on the rate of decline in leaf quality. Also, more specialist species are constrained to their own host plant, whereas generalist species can feed on a broader range of host species and thus have a higher chance of dispersing to another host plant where there is food available and, in general, having a less-sharp peak in their fitness curve.

The shape and position of the fitness peak vary from place to place and from year to year. Hence, insect herbivores need to adapt to both spatial and temporal variation in the phenology of their host trees.

Adaptation to Spatial Variation in Plant Phenology

Tree phenology varies between areas, within areas, and even at the scale of adjacent trees (Crawley and Akhteruzzaman 1988; Van Dongen et al. 1997). This variation is predictable, as trees are consistently early or late in subsequent years (e.g., Van Dongen et al. 1997). Hence, herbivore species can and need to adapt to this spatial variation to achieve synchrony across their habitat. If a trait, such as egg hatch, is genetically determined and has sufficient genetic variation, than we expect that through selection, a close synchrony with the host plant can be maintained. Many traits have some kind of genetic basis, and these include phenological phenomena (e.g. Lill 2001). On a spatial scale, genetic differences have been found for several species between different geographic populations (e.g. Peterson and Nilssen 1998; e.g. Tammaru et al. 2001), between populations on different host plants (e.g., Du Merle 1999), and within populations (e.g., Akimoto 1998; Van Asch et al. 2006). Even on a small spatial scale, genetic differences exist between populations that occur closely together (e.g., Komatsu and Akimoto 1995; e.g., Van Dongen et al. 1997; Mopper et al. 2000). In such a case, populations differ between individual host plants; the herbivores have become adapted to their specific host plant. This process is called adaptive deme formation (for an overview, see, e.g., Van Zandt and Mopper 1998; Kawecki and Ebert 2004; for an overview, see, e.g., Mopper 2005). Limited dispersal is one of the conditions that needs to be met for adaptive deme formation to occur because gene flow can potentially prevent adaptation. Mopper (Mopper 2005) states that feeding mode appears especially to be an important factor in determining whether adaptive deme formation can occur because it occurs more in internally feeding species than in externally feeding species. She argues that synchrony with the host plant is even more crucial for internally feeding species; thus selection on synchrony is even stronger, and deme formation is more likely.

A nongenetic way in which some degree of synchronization with individual host trees also may occur is via maternal effects. Maternal effects are a special form of phenotypic plasticity that act across generations (Mousseau and Dingle 1991), and they are increasingly

recognized as a major contributor to adaptation (Mousseau and Fox 1998). Known maternal effects include the number and size of offspring (Mousseau and Fox 1998), the amount of resources invested by the parents (Rossiter 1996), and the determination of diapause in insects (Mousseau and Dingle 1991) and dormancy in plants (Roach and Wulff 1987) in response to the photoperiod experienced by the mother. If the feeding conditions of a mother not only affect her fecundity, but also the generation time, this may be a way to achieve synchrony with an individual host plant. Feeding conditions of the mother do indeed influence the time until egg hatch of her offspring in winter moths (M. van Asch, unpublished data). Maternal effects may be a good mechanism to achieve synchrony with the host plant after dispersal to another plant with a different phenology without the need for any genetic differences.

Adaptation to Temporal Variation in Plant Phenology

Spatial variation in host plant phenology is not the only kind of variation that an insect herbivore may encounter; there is also a large temporal variation (i.e., year-to-year fluctuations) in the phenology of the host plant. Insect herbivore phenology should follow these fluctuations in host plant phenology, which are mainly results of variations in environmental conditions (temperature) between the different years (see above). Insect phenology is indeed affected by environmental conditions; i.e., phenology is phenotypically plastic (Scheiner 1993). Researchers often present the exact relationship between phenotype (i.e., different hatching time of eggs) and environment (i.e., temperature) as a reaction norm (Figure 4) where the elevation reflects the mean phenotype across environments, and the slope reflects the sensitivity of the phenotype for the environmental variable (Scheiner 1993). This phenotypic plasticity of the herbivores should be sufficient to track the temporal variation, as natural selection will have selected for a temperature sensitivity of the herbivores similar to the effect temperature has on tree phenology, despite that the mechanism underlying plasticity (see above) may be different for the herbivores.

To achieve closer synchrony with the host plant, some species need to change their reaction norm not in the mean phenology across environments (elevation of the reaction norm), but in their temperature sensitivity (slope of the reaction norm) (Figure 4). Changes in intercept only lead to an average increase of synchrony, but the asynchrony can still be considerable in individual years. Changes in temperature sensitivity (slope) are much harder to achieve than changes in the mean phenology across environments (elevation), which may reflect both genetic correlations across environments (Etterson and Shaw 2001) (Wijngaarden and Brakefield 2001) and physiological limitations.

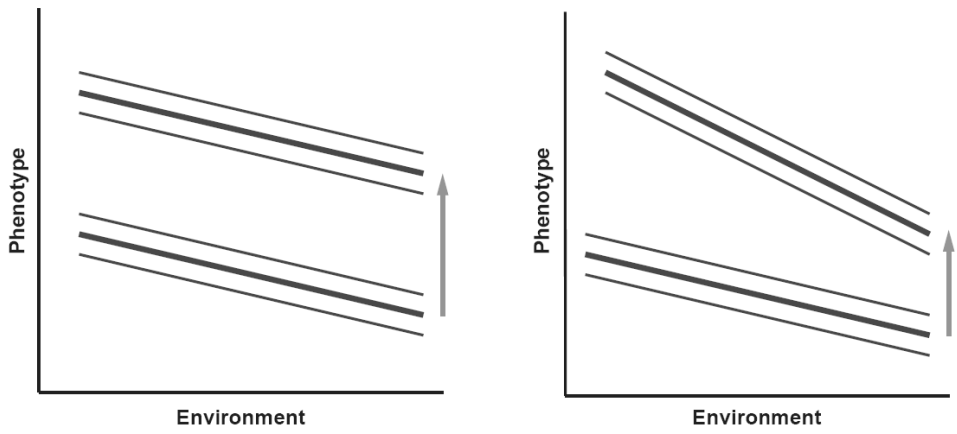


Figure 4 Shifts in phenotypic responses to different environmental conditions under selection. (Left panel) Change in mean response (elevation) but not in phenotypic plasticity (slope). (Right panel) Change in mean reaction norm and in phenotypic plasticity. Thick lines represent a mean reaction norm; thin lines represent the variation around the mean.

Population dynamics

Synchronization, Population Dynamics, and Outbreaks of Pest Species

Synchronization with the host plant has major fitness consequences for many insect herbivores, and we therefore also expect it to affect their population dynamics. Many leaf-feeding herbivores in spring have strongly fluctuating population densities, such as *C. occidentalis*, *E. autumnata*, *L. dispar*, *O. brumata*, and *T. viridana*. These species are outbreak species because they may completely defoliate their host trees at peak densities, causing severe damage. Many other herbivore species vary to a much lesser degree in their density, whereas other species experience regular outbreaks in some regions but not in others. In this section, we discuss the way synchrony with the host plant may affect population densities.

Population Fluctuations and Synchrony

As early as the 1970s, researchers suggested synchrony with the host plant as the major explanation of population fluctuations of outbreak species such as *O. brumata* (Varley and Gradwell 1968; Feeny 1976). The population dynamics of *T. viridana* are also thought to be driven by synchrony (Hunter 1990, and references therein). If herbivores are well synchronized with their host plant, many first instar larvae survive and population density increases, whereas if synchrony is poor, few larvae survive or they have a much-lower fecundity and decreased population size.

However, studies demonstrating the relation between population fluctuations and synchrony with the host plant are relatively scarce and the results inconsistent. Synchrony is important for the population dynamics of western spruce budworm, *C. occidentalis* (Thomson et al. 1984), but synchrony is much less important for *L. dispar* (Hunter 1993). This may be explained by the relatively high resistance of *L. dispar* to starvation. Winter moth population fluctuations on Sitka spruce, *P. sitchensis*, could also not be explained by

synchrony (Hunter et al. 1991), but winter moth fitness when feeding on Sitka spruce is probably less dependent on synchrony than when feeding on oak (*Quercus robur*). Watt & Woiwod (1999) assumed that most moths feeding in spring are more sensitive to phenological asynchrony than later-feeding species and are therefore potentially more prone to population fluctuations. However, they failed to find a correlation between feeding period and population fluctuations.

Synchrony has long been assumed to play a major role in the population dynamics of insect herbivores, but the number of studies actually studying this relationship directly is relatively low and not all can confirm a major role. However, even if it is not the only important factor, synchrony does likely play a major role in population fluctuations.

Population Fluctuations and Climate

Because of the limited evidence for a directly measured effect of synchrony on population fluctuations, we now look at more indirect evidence based on climatic variables. Population densities are often reported to correlate with climatological variables, such as the North Atlantic Oscillation (NAO) in Europe. Outbreaks of *E. autumnata* are an example of this correlation (Klemola et al. 2003). The NAO may not only influence mean air temperature, but it may also influence precipitation and other weather variables (Hurrell et al. 2001). The effect temperature has on population densities may well work via processes influencing synchrony, such as mistiming in warm or cold springs, increased starvation risks for herbivores when it is warm, or a faster buildup of leaf defense chemicals. Another factor that may affect population densities is sunspot activity. Population fluctuations of several moth species in Scandinavia correlate with sunspot activity (Ruohomaki et al. 2000; Selås et al. 2004), including both *E. autumnata* and *O. brumata*. Sunspots may affect population densities via a direct effect of UV-B on insect performance, with high levels of UV-B radiation being harmful (for an overview, see, e.g., Julkunen-Tiitto et al. 2005). However, an effect of the sunspot activity likely works indirectly via an effect on the host plant. Trees need to put more resources into protection against UV-B radiation, and they may then put fewer resources into herbivore defenses, or insects may somehow benefit from an increased flow of resources toward the leaves (Selås et al. 2004). One study that seems to support this found that winter moths prefer leaves grown under elevated UV-B to those grown without elevated UV-B (Lavola et al. 1998). However, many other studies found that plants grown under high UV-B radiation are of poorer quality for herbivores (Haukioja 2005). As an alternative mechanism, an increase in defensive compounds in the host plant may lead to an increased resistance to parasites (Haukioja 2005). An alternative hypothesis for the correlation with sunspots is that sunspots directly affect the temperature as experienced by the herbivores, but not so much the temperature as recorded by weather stations (which measure temperature in the shade). In that case, the indirect effect of sunspots may be via temperature and from the synchrony between host plant and insect herbivore.

Population Regulation and Synchrony

Population regulation may come about via other processes than the ones that are responsible for population fluctuations (Turchin 1999). Predation, parasitism, viruses, and pathogens are all potentially important factors regulating population densities. Varley & Gradwell (1960) demonstrated that pupal predation is one of the factors regulating *O. brumata* populations. Outbreaks of pest species such as *E. autumnata* (Tanhuanpää et al. 1999), *L. dispar* (Liebhold et al. 2000), and *O. brumata* (Raymond et al. 2002) have been associated with lack of pupal predators (but see Hunter et al. 1991, which shows that nutrients, pupal predation, and synchrony all fail to explain population outbreaks of *O. brumata* on *P. sitchensis*). Although larval parasites appear to play only a relatively minor role in England (Varley and Gradwell 1960), they (e.g., the tachinid fly, *Cyzenis albicans*) have been effective biocontrol agents against *O. brumata* in the United States and Canada (Roland 1994; Roland and Embree 1995). After a first decline caused by the larval parasites, the low *O. brumata* densities were maintained by pupal predators. Many passerine birds feed their young on leaf-eating caterpillars in the spring. Preventing birds from feeding on the larvae increases larval survival (Strengbom et al. 2005), whereas increasing the number of birds can reduce larval densities in orchards (Mols and Visser 2002). Larval predation by birds is an important factor in maintaining low *E. autumnata* densities (Tanhuanpaa et al. 2001). In summary, predators, parasites, and pathogens may affect the population densities of insect herbivores, and synchronization, although it may be the prime mortality factor, seems not to be involved in population regulation.

However, synchrony may have more-subtle effects on population regulations via a delayed effect of herbivory, either on tree phenology or on the chemical composition of the leaves in the following year (Haukioja and Neuvonen 1988). Artifical defoliation can reduce the performance of insect herbivores the following year (Haukioja 2005). After heavy defoliation by *E. autumnata*, *B. pubescens* development is delayed in the following year (Kaitaniemi et al. 1997), and this delay could potentially reduce the synchrony between the trees and the larvae. However, almost-complete defoliation only is sufficient to cause a delay in bud bust the following year (Kaitaniemi et al. 1997). Herbivore densities required to achieve this level of defoliation are so high that egg hatching in the following year is also delayed, thereby annihilating any effect of a delay in bud opening. Another example is the jack pine (*Pinus banksiana*), which suffers from heavy defoliation in the northern United States by the pine budworm (*C. pinus pinus*) (Lepidoptera: Tortricidae). The budworm's survival depends on the abundance of pollen cones. After heavy defoliation, the trees produce fewer cones, thereby reducing the survival of first instar larvae (McCullough 2000). Thus the response of the tree to defoliation, owing to synchrony of the phenology of the herbivores with their trees, may lead to mistiming in the following year, reducing population numbers.

C**hanges in the environment**

Global Climate Change

Climate is changing and will continue to change: Global predictions for the next century range from an increase of 1.5º C to 5.5º C (IPCC 2001), whereas these changes may be considerably larger on a local scale. The expected rate of change is much faster than any other changes previously experienced, and the expected temperatures are usually well outside the range of temperatures now experienced by organisms in the same place. Weather patterns are also predicted to become more variable (Easterling et al. 2000). In addition, climate changes may not be uniformly distributed across the whole year and across regions; whole climate patterns may change. Because phenology is in many cases temperature dependent, climate change will also lead to changes in phenology. As temperatures are increasing, the phenology of both the insect herbivore and its host plant is expected to advance. Several studies have described the effects of climate change on species (e.g. Roy and Sparks 2000; Bale et al. 2002; Parmesan and Yohe 2003; Root et al. 2003), which include range shifts and advances of phenology in spring. If both the host plant and the herbivore are affected to the same degree, phenotypic plasticity maintains close synchrony. However, the phenology of the insect herbivore and its host plant will not necessarily shift at the same rate. Thus climate change can potentially lead to a decrease in synchrony or a mismatch between different trophic levels (Bale et al. 2002; Stenseth and Mysterud 2002; Visser and Both 2005). The winter moth (*O. brumata*) in the Netherlands is an example of this (Visser and Holleman 2001) (but see (Buse et al. 1999) for the effect of climate change on winter moths in England). Currently, winter moth eggs tend to hatch before fresh oak (*Q. robur*) leaves, their main food source, become available to the larvae. In some years more than 90 % of the eggs hatch before the first oak buds open. In the past 25 years, early spring temperatures have increased, whereas winter temperatures have not. Visser & Holleman (2001) argue that this change in temperature pattern is responsible for the decreased synchrony between winter moth and oak. In this example, phenotypic plasticity alone is not sufficient to maintain synchrony because climate change presumably disrupts the correlation between the environmental cues used by the winter moth and oaks. To restore synchrony, the response of the moths to temperature needs to change: The eggs need to become less sensitive to temperature so that they hatch later, at the time of bud opening (Van Asch et al. 2006). If climate change does lead to asynchrony between an insect herbivore and its host plant, synchrony can be restored in three different ways.

First, synchrony can be restored via a response to the directional selection. If there are severe fitness consequences of feeding (or, for example, egg laying) outside the optimal period set by the phenology of their host, there is a directional selection on herbivore phenology, which may lead to adaptation. However, if selection pressures become strong, this can lead to a rapid response but may lead to population extinction as the reproductive output declines strongly. A response to selection is possible only if sufficient genetic variation for that particular trait exists. Because the predicted increase in temperature is much larger compared with previous temperature fluctuations, even if there is genetic

variation, it may not be enough to match the differences needed. Even with sufficient genetic variation, sometimes no adaptation occurs (Van Asch et al. 2006), as genetic correlations between traits may prevent adaptation (e.g., Etterson and Shaw 2001).

Second, an herbivore may shift to a different host species with a more-suitable phenology. This is especially true for generalist species. Specialist species are dependent on their own specific host plant; shifting to a new host is much harder for them.

Third, if the circumstances are no longer suitable and the herbivore cannot adapt to its changing environment, then the local population goes extinct. This may be a rather sudden process. Powell and Logan (2005) show, using mountain pine beetles as an example, that under climate change changes in voltinism are likely to happen suddenly rather than as a gradual process. Populations of univoltine species dependent on timing with their host plant, occurring near their thermal boundaries, may go suddenly extinct. However, circumstances elsewhere may possibly become favorable where they were not before. If the herbivore disperses, it may be able to establish itself in such new locations, leading to, for instance, range shifts (Parmesan and Yohe 2003). However, the relationship between climatological variables (e.g., temperature and photoperiod) changes with geographic location, leading to problems such as with diapause induction (Musolin and Numata 2003).

Environmental change affects not only the start of feeding in spring, it also affects the further development of both herbivore and host. An increase in CO2-levels changes the nutritional suitability of the leaves for the herbivores. A higher CO2-concentration leads to a reduction in nitrogen content and it may lead to an increase in defensive chemicals (Lincoln 1993). Insect herbivores either increase their consumption rate to make up for decreased nitrogen availability, or they reduce their growth rate (Lincoln 1993). Under increased CO2-levels *O. brumata* is able to consume more *Q. robur* leaves due to a reduction in leaf toughness (Buse et al. 1998), while *L. dispar* has a similar pupation weight but needs a longer time to develop due to an increase in condensed tannins (Lindroth et al. 1997).

The effects of climate change on synchrony can be diverse, and even if climate change leads to asynchrony, there are several options to adapt to the changed phenology of host plants. However, many species will undoubtedly not be able to adapt to their changed environment, and these may be seriously affected.

Outlook

Synchrony of an herbivore's phenology with that of its food is clearly crucial to many leaf-feeding Lepidoptera and to many other species (Visser and Both 2005). The way in which any organism achieves such synchrony in a fluctuating environment stresses the need for more mechanistic approaches at both trophic levels. Even though physiological models do exist, they are not commonly used in ecological studies. A more-integrated approach would lead to a better understanding of the processes involved in achieving and maintaining synchrony between different trophic levels. Also, if we need to extrapolate the models to

future conditions under climate change, which may be well outside the current range, better physiological models are of crucial importance.

Understanding synchrony between trophic levels has become particularly pressing in view of climate change. Because of interactions between species, small effects on phenology may lead to considerable deleterious effects if the synchrony between trophic levels is disrupted. Not only is the amount of change needed to restore synchrony important, but so are the rate of these changes and the possible consequences these changes have on the population level. Understanding the underlying mechanisms and the extent to which they are genetically determined is crucial in assessing the evolutionary potential for species to deal with worldwide climatic changes.

Acknowledgments

We would like to thank Kate Lessells, Arjen Biere and two anonymous referees for helpful comments on the manuscript. Margriet van Asch was supported by ALW research grant 812.04.009 from the Life Sciences Foundation.

Chapter 3: Modeling *Operophtera brumata* egg phenology: nonlinear effects of temperature and of developmental stage on development

Margriet van Asch[1], Gerdien de Jong[2] and Marcel E. Visser[1]

[1] Netherlands Institute of Ecology (NIOO-KNAW), Centre for Terrestrial Ecology, P.O. Box 40, 6666 ZG Heteren, The Netherlands
[2] Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Abstract

Accurately predicting when a species reaches a certain life stage is crucial for controlling pest species, as well as for predicting phenological changes due to environmental changes. Often simple, linear degree-day models give a good correlation with observed data. However, degree-day predictions are only valid for the populations and temperature patterns that were used to estimate the model parameters. If temperatures fall outside the temperature range, development rate is no longer a linear function of temperature, as assumed in the degree-day models. Moreover, when interested in for instance the genetic variance in temperature response, it is crucial to have a more mechanistic model. We use here a physiologically based, nonlinear model to describe winter moth (*Operophtera brumata*) egg hatch. This model allows non-linearity of development rate with temperature. Development rate increases with increasing developmental stage, although we did not find evidence of a true diapause. Incorporating this effect temperature-independent into the model greatly improved its predictive value under natural conditions. How developmental stage and temperature interact in development needs further study.

Introduction

The moment when an organism reaches a certain stage of the life cycle is important for many species. Especially in climates with strong seasonality development can only occur if the environmental conditions are suitable. Abiotic conditions such as temperature or water availability as well as biotic conditions such as food availability may vary greatly within a year. How organisms deal with this seasonality has been the focus of many studies (e.g., Tauber and Tauber 1981; Wolda 1988; Van Asch and Visser 2007). In order to understand how species can maintain their seasonality, we need to understand the mechanism that determines when egg hatch takes place, and how a species' eggs manage to hatch at the proper moment in time each year. Moreover, being able to predict accurately the emergence time of a species can play a major role in controlling pest species (e.g., Logan *et al.* 2003). Understanding the mechanisms determining egg hatching time is also important when trying to predict effects of environmental change on phenology.

In ectotherm species such as insects, development rate depends on temperature, as physiological processes go faster at a higher temperature. Models describing insect development consequently mainly look at the relationship between temperature and development rate. Degree-day models are very commonly used in ecological studies describing development rates. Degree-day models assume a linear relationship between development rate and temperature. Degree-day models also assume a threshold value below which no development takes place. Examples of species in which degree-day models provide a good approximation of temperature-dependent development rate include many Lepidoptera such as Geometridae (e.g., *Operophtera brumata*) (Embree 1970; Watt and McFarlane 1991; Dewar and Watt 1992; Buse and Good 1996), Tortricidae (Lysyk 1989) and gypsy moth (*L. dispar*) (Andresen *et al.* 2001). Degree-day models generally give a good fit since the relationship between development rate and temperature is more or less linear at intermediate temperatures and the temperature sum for development is about constant. However, at the more extreme temperatures development is usually not a linear function of temperature (e.g., Huey and Kingsolver 1989). Therefore degree-day models are only valid at an intermediate temperature range. It may well be that when the climate changes, temperatures fall outside the linear part of the development curve, or that temperature patterns change. If so, the linear (degree-day) models cannot be used to predict a species' response under climatic change. Other models describing nonlinear relationships between temperature and development rate are called for; one such model is the physiological Sharpe-Schoolfield model (Schoolfield *et al.* 1981) (Wagner *et al.* 1984).

Winter moth (*Operophtera brumata*) egg hatching has been relatively well studied (Embree 1970; Kimberling and Miller 1988; Visser and Holleman 2001). Winter moths have an annual life-cycle; female moths lay their eggs in the late autumn in the canopy of a tree, and these hatch the following spring. The moment of egg hatching is crucial- as only young leaves are suitable for feeding (Feeny 1970; Tikkanen and Julkunen-Tiitto 2003; Van Asch *et al.* 2007). The timing in the food chain oak-winter moth-great tit has been

extensively studied (Visser *et al.* 1998; Buse *et al.* 1999; Tikkanen and Lyytikainen-Saarenmaa 2002), also with respect to climate. Buse and Good (1996) found that egg hatch advanced at an elevated temperature; however, this response matched the response of the oak trees in their study population. In contrast, Dutch winter moths seem to respond more strongly to temperature changes than Dutch oak trees, potentially leading to increased mistiming under climate change (Visser and Holleman 2001). However, the study of Visser and Holleman was mainly based on a correlational model. Although this model gave a relatively good fit to their data, we need to understand more fully what is happening in the eggs before extrapolating to other temperature patterns. Embree (1970) already showed that both at low and at high temperature the relationship with development rate becomes nonlinear in the winter moth.

A main assumption of both the linear degree-day and the nonlinear models modeling development rate as a function of temperature is that development rate is homogeneously determined during development. The processes are assumed to depend only on temperature; model parameters do not change due to age or feeding status of an organism. If such homogeneity is not the case, such as in species with a diapause, these models cannot give an accurate prediction.

Diapause, a hormonally induced state of very low metabolic activity, increases the resistance to unfavorable environmental conditions (Tauber and Tauber 1981). Diapause can also serve to prevent development in the wrong season, such as egg hatching in the autumn in a species that overwinter as eggs. Evidence exists that suggests that some winter moth populations do have an egg diapause (Kimberling and Miller 1988; Visser and Holleman 2001). Winter moth studies using a degree-day model only start counting the degree-days after a certain date, by which date diapause presumably had been terminated (e.g., Buse and Good 1996). Alternatively, they may include some kind of chilling requirement in the model (Kimberling and Miller 1988; Visser and Holleman 2001) . However, most of these studies are again based on correlational data. These models might give a good fit because a diapause phase actually exists, but diapause is not necessarily the only explanation of the improved fit. Degree-day models assume that no development takes place below the threshold temperature. However the seemingly faster development after a cold period could be explained too if some development took place at very low temperature, albeit at a very low rate. Alternatively, correlations between temperatures during different periods of development could cause the correlation between cold period and thermal requirements afterwards.

The aim of this study was to create a more physiological, nonlinear model that describes winter moth egg hatch. To do this, we need to know the exact development rate in response to temperature. We also check whether development rate changes over time, and whether cold periods have an effect on egg hatch (i.e., diapause termination). We can then compare this model's predictions with those of existing models describing winter moth egg hatch and compare these with real data on egg hatching.

Methods

Origin of the eggs

Prior to the experiment we caught female winter moths in Oosterhout (05°50' E, 51°55' N), the Netherlands, using insect traps on mature oak trees. Traps were checked three times a week. Females were put individually into plastic containers, provided with a roll of tissue paper to lay their eggs on and then placed in an outdoor insectarium. After two days under outside conditions the eggs were transferred to a climate chamber (Sanyo Incubator MIR-553) at 5 °C, were the eggs were kept for 30 days until the start of the experiment.

We performed experiments in two consecutive years (2004 and 2005). In 2004 we used females caught in the last two weeks of November (mean catching date 24 November 2003) and the experiment started 30 days after the mean catching date. In 2005 we used females with the same catching date, and several replicates with different catching dates (19, 24, 29 November and 8 December 2004) and in all cases the experiment started exactly 30 days after catching the females.

Mean development rate at constant temperatures

We measured mean development rate by putting eggs at different, constant temperatures, and then determining egg hatching dates.

Eggs from each female were divided over four different temperature treatments (split-brood design), thus ensuring that there were no genetic differences between eggs in each treatment. At the start of the experiment these eggs were put at their experimental temperature. In 2004 experimental temperatures were 5, 10, 15 and 20 °C. As there were eight experimental treatments in 2005, the eggs from each female were put in one of two series of temperature treatments: they were kept either at 4, 6, 15 and 23 °C or at 5, 7.5, 10 and 12.5 °C.

Median egg hatching date was determined for the eggs from each female at each temperature treatment. Each replicate at each temperature consisted of eggs from 15 females. In 2005 we caught females on four different dates (15 females on each date), these are treated as replicates. Average development time for all females at each replicate was then determined.

Parameter estimation nonlinear model

We fitted our data to a nonlinear model with a biophysical background (Sharpe and DeMichele 1977; Schoolfield *et al.* 1981; Wagner *et al.* 1984; Wagner *et al.* 1991). This model assumes that development rate is regulated via activation and inhibition of enzyme activity. Development rate (r)(time^{-1}, days) at temperature T (°K) is then given as:

$$r(T) = \frac{\frac{\rho T}{T_{ref}} \exp\left(\frac{H_A}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right)}{1 + \exp\left(\frac{H_L}{R}\left(\frac{1}{T} - \frac{1}{T_L}\right)\right) + \exp\left(\frac{H_H}{R}\left(\frac{1}{T_H} - \frac{1}{T}\right)\right)} \qquad (1)$$

With:

ρ = development rate (time^{-1}) at the reference temperature T_{ref}

T_{ref} = reference temperature (°K) where no enzyme inactivation occurs; here T_{ref} = 12.5 °C = 285.5 °K

H_A = activation enthalpy (J/mol) of the reaction catalyzed by the rate controlling enzyme

T_L = temperature (°K) at which half of the enzyme molecules are active, while the other half are in the low temperature inactive state

H_L = enthalpy change (J/mol) associated with low temperature inactivation of the enzyme

T_H = temperature (°K) at which half of the enzyme molecules are active, while the other half are in the high temperature inactive state

H_H = enthalpy change (J/mol) associated with high temperature inactivation of the enzyme

R= universal gas constant (=8.314 J K^{-1} mol^{-1}).

We used a nonlinear procedure in SAS (v8) to estimate the parameter values. Development rate is given as development time^{-1} (days). Since the eggs had already developed before the start of the experiment, we needed to correct for this development. Based on experimental data (see below), we assumed that in that period already 5% of total development had occurred. Mean development rate at the experimental temperature is then given by (1-0.05)/(total development time-30). Because at high temperatures mortality was high, our temperature range was not large enough to reliably estimate the high temperature inactivation, we therefore used the four parameter version of the model instead:

$$r(T) = \frac{\frac{\rho T}{T_{ref}} \exp\left(\frac{H_A}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right)}{1 + \exp\left(\frac{H_L}{R}\left(\frac{1}{T_L} - \frac{1}{T}\right)\right)}$$

(2)

Assumptions underlying the model

The main assumption of the model we used is that development rate is homogeneous during development, that is, that the parameters of the model are identical between stages and independent of egg age. Development will be identical at identical temperature whatever the time of the year. This assumption need not be valid. Winter moths may experience a diapause with greatly reduced metabolic rate and thus development. Some species (e.g., *Tortrix viridana*, (Du Merle 1999)) need to experience a cold period in order to break

diapause. To check whether winter moth eggs need to experience a cold period before development can start we kept some female moths at 15 °C and then kept their eggs at 10, 15 or 20 °C. Eggs hatched normally in all three cases. Even so, development rate may vary over time, as seems to be the case with gypsy moths (Gray *et al.* 1995; 2001), whose respiration rate changes gradually. To estimate the effect of a cold period, as well as to estimate the change in development rate over time eggs were given a different cold treatment. Eggs were given a four-week cold treatment, either in January or February. Outside the cold treatment the eggs were kept at a constant 10 °C, during the cold treatment eggs were kept at 3, 4, or 5 °C. Eggs from each female (n=20) were divided over the two cold treatments, but eggs from different females were used at the different temperatures. Analyses were done using a mixed procedure in SAS (v8) with period of cold and temperature as explanatory variables; females were nested within temperature treatment and treated as random factors.

Another assumption of the model we used is that egg hatch is solely temperature dependent. Eggs could potentially also use photoperiod and/or some direct chemical cues from the oak buds. No evidence suggests that eggs do use chemical cues (L. Holleman, unpublished data). To test for effects of photoperiod, we performed a split-brood design (2001) with two temperature patterns and two light regimes. In the autumn (2000) females were caught in Oosterhout (05°50' E, 51°55' N). Prior to the experiment the eggs were kept under outside conditions. At the start of the experiment (12 December 2000) eggs were divided over four treatments, each in a separate climate chamber. Two temperature treatments were given (Visser and Holleman 2001), each mimicking a specific year, a 'warm' year (1999, mean temperature 6.2°C) and a 'cold' year (1973, mean temperature 4.5°C). A three-phase cycle was given, with 6 hours at the daily minimum, 12 hours at the mean of the daily average and daily maximum temperature and 6 hours at the daily average temperature in that specific year. This treatment keeps any natural pattern that may exist in a given year intact, rather than giving artificially constant temperatures. For each temperature treatment two light regimes were given. Both light regimes followed the natural increase in day length. One of the light regimes was two weeks advanced relative to the day length at a given date, while the other was two weeks delayed. This led to a maximum difference in day length of two hours in mid-March. Light regimes were changed twice a week, temperature three times a week. Temperature in the climate chambers was measured every 15 minutes using HOBO-temperature loggers. Next spring median egg hatching date was determined.

Validation of the model

To validate the model, we compare the values predicted by the model with the actual observed hatching dates (1995-2005). These dates were obtained by catching (at least 20) females in Oosterhout (05°50' E, 51°55' N), the Netherlands. The eggs these females laid were then kept in an outdoor insectarium. In spring, the eggs were checked three times a week for emerged caterpillars, and median hatching date was determined for each brood.

Next, we compare the nonlinear model with a linear degree-day model. The number of degree-days necessary before egg hatch we calculated from our experimental data (DD=395, above a threshold value of 3.9 °C). In addition, we also compare it with an existing model using both degree-days and frost-days (Visser and Holleman 2001). In this model the number of degree-days needed for egg hatch decreases with the number of days when the temperature came below 0 °C. We could not separately parameterize this model on our experimental data (as temperature treatments did not include temperatures below 0 °C). Therefore we use the values published previously (Visser and Holleman 2001). However, the parameter values they used were part of the same dataset we also use here (observed hatching dates 1995-1999). Analysis were done in SAS (v8), and compared the (absolute) difference of a model with another model or the observed values.

Results

Winter moth egg hatching is strongly temperature dependent, and the nonlinear model describes development rate well (figure 1). Parameter estimates are as follows: at 12.5 °C (285.5 K) we assumed that no enzyme inhibition occurred. At this reference temperature the development rate ($r_{12.5}$) was 0.0155 day^{-1}. Activation enthalpy (H_A) was 62 kJ mol^{-1}, and at 3.9 °C (276.9 K) half of the enzyme molecules are inactive due to low temperature inactivation (T_L). A linear degree-day model overestimates development rate at low temperatures, and underestimates development rate at higher temperatures (figure 1).

The main assumption of both the physiological model and degree-day models is that development rate remains homogeneous throughout the stage it describes. This does not seem to be the case for the winter moth eggs (figure 2a). A cold period in February affected development time more than a cold period in January ($F_{1,40}$=127.1; p<0.001). Some development did still occur during the cold period, as development time was longer if the temperature was lower ($F_{1,40}$=7.86; p<0.001). Although the precise inhomogeneity in development rate over time still needs further study, we want to check here what consequences incorporating such an stage effect into the model will have. Therefore we assume that development rate increases linearly over time. We estimate the effect of cold in our late cold treatment relative to the effect in our earlier cold treatment. We then express the development rate as a function of developmental stage rather than time (figure 2b).

Another assumption of both models is that egg hatch is solely temperature dependent. Photoperiod did not affect winter moth egg hatch. Although longer day length seemed to advance egg hatching at the lower temperature, this effect completely disappeared after correcting for temperature differences between the photoperiod treatments (figure 3) ($F_{1, 58}$=1.62, p=0.2).

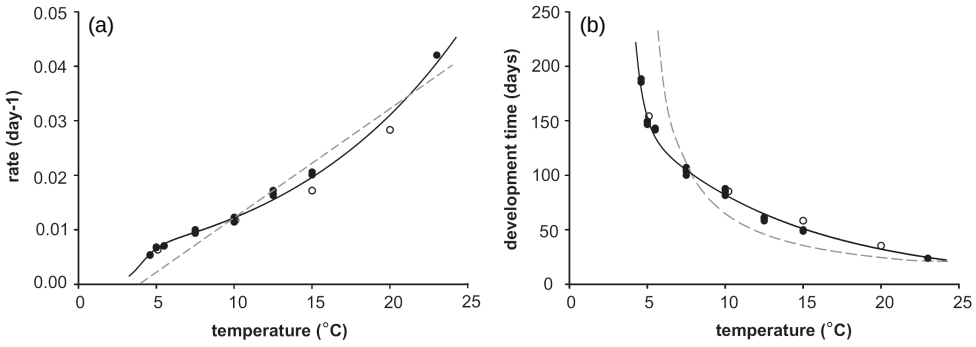
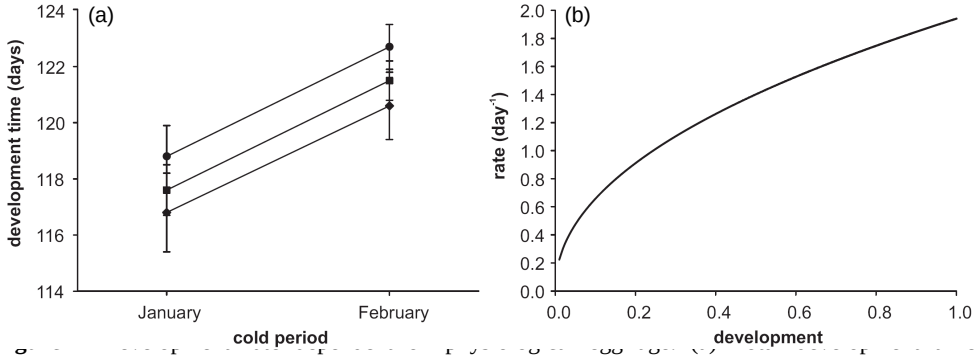


Figure 1 (a) Mean development rate ($^{1}/_{\text{days until egg hatch}}$)(±s.e.m.) and (b) mean development time (days)(±sem) at different constant temperatures (°C) in two consecutive years (2004 open symbols, 2005 filled symbols) for eggs from female winter moths caught in Oosterhout. Multiple values represent replicates with a different laying date (2005). The solid line describes egg hatch using the nonlinear, four parameter model, and the dotted line describes egg hatch using a linear degree-day model (egg hatch at 395 DD, threshold value 3.9 °C).

We can now compare hatching dates predicted by the two models with actual, observed hatching dates (figure 4). A degree-day model predicts egg hatch too late in all years. This is due to the underestimation of development rate at low temperature. On the other hand, the physiological model assuming a homogeneous development rate predicts egg hatch too early in almost all years. However, including an effect of development stage on development rate greatly improves the predictions. Overall, the predictions from both degree-day models (degree-day model parameterized from our experimental data: $F_{1,20}$=40.3, p<0.01; degree-day model including an effect of cold (Visser and Holleman 2001): $F_{1,10}$=6.7, p=0.03) and the physiological model assuming constant development rate ($F_{1,20}$=4.8, p=0.04) significantly differ from the observed egg hatching dates. Only the model incorporating an increase in development rate over time predicts egg hatch well ($F_{1,20}$=0.5, ns).



(days)(mean ± s.e.m.) for eggs from female winter moths caught in Oosterhout, experiencing a four-week cold period in January or February. Temperature during the cold period was 3 (circles), 4 (squares), or 5 °C (diamonds). (b) Assumed effects of physiological egg age on development rate used in the model. Development rate is expressed relative to the mean development rate at 12.5 °C (=0.0155 day^{-1}), and under the assumption that the relationship between development time and egg age in (a) is linear.

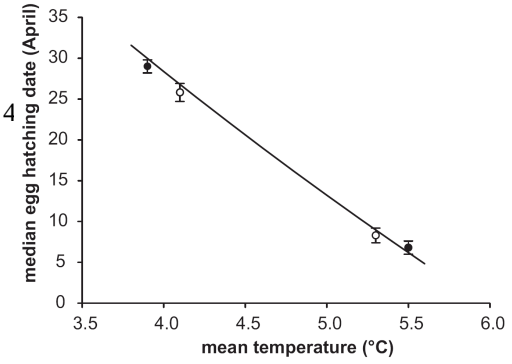
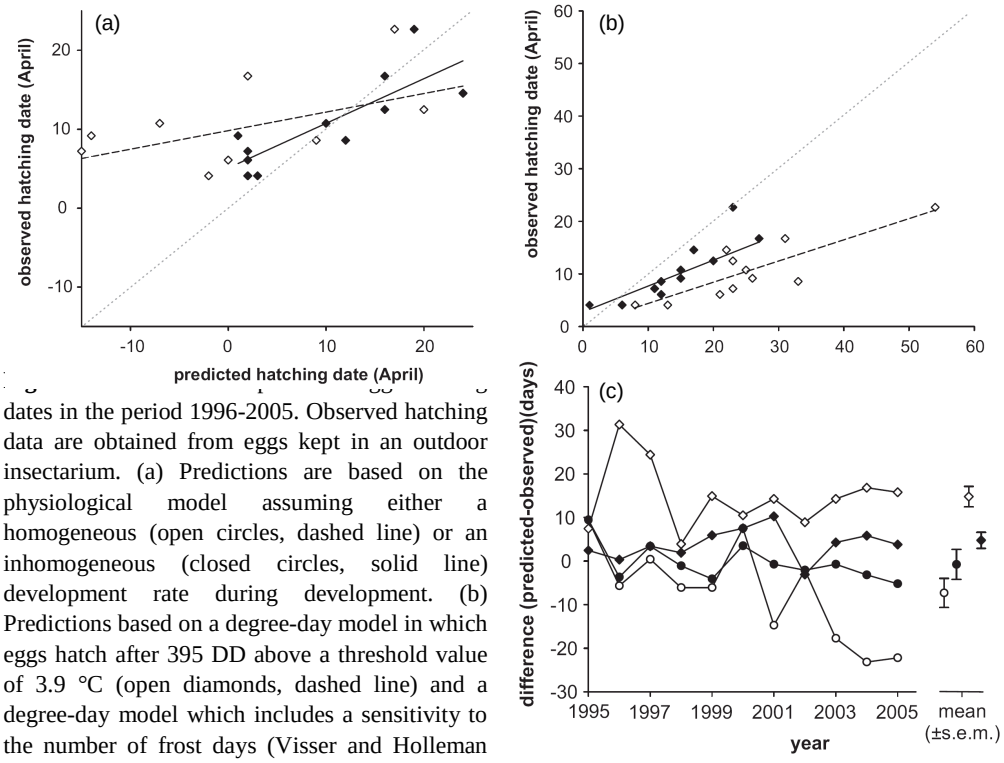


Figure 3 Effect of temperature and day length on median egg hatching date (April) (mean ± sem). Filled symbols represent short day length, open symbols represent long day length, solid line represents the prediction from the model. Temperature is the mean daily temperature from December 10th until mean median egg hatching in each treatment.

Discussion

Winter moth egg development rate can be described very accurately under experimental temperatures by the physiological model. Although a linear degree-day model fitted the data reasonably well at the intermediate temperature ranges, it underestimated the development rate at low temperatures. However, under more natural conditions both the physiological model and the degree-day model did not give a good description of the data. The most likely explanation is that the effect of temperature on development rate itself changes during development, whereas both models assume that development rate remains



dates in the period 1996-2005. Observed hatching data are obtained from eggs kept in an outdoor insectarium. (a) Predictions are based on the physiological model assuming either a homogeneous (open circles, dashed line) or an inhomogeneous (closed circles, solid line) development rate during development. (b) Predictions based on a degree-day model in which eggs hatch after 395 DD above a threshold value of 3.9 °C (open diamonds, dashed line) and a degree-day model which includes a sensitivity to the number of frost days (Visser and Holleman 2001) (closed diamonds, solid line). (c) Yearly and mean (± s.e.m.) predictive values for all four

models. Lines in (a) and (b) are regression lines, with equation: 1) y=0.23x+0.12 (R^2=0.29), 2) y=0.62x+4.3 (R^2=0.71), 3) y=0.40x+0.35 (R^2=0.70), 4) y=0.49+2.7 (R^2=0.77). Note: the degree-day model including the number of frost days is a previously published model (Visser and Holleman 2001) whose parameter values were based on the 1996-1999 data, the regression line in (b) and mean values in (c) for this model are therefore based on the 2000-2005 data only; all other models were parameterized using our experimental data.

homogeneous, at given parameter values for all temperatures. Winter moth egg hatching is stronger delayed when experiencing a cold period later in their development. Incorporating such an effect into the model improved the fit with the data obtained under natural temperature regimes. At present, the exact change in temperature responsiveness during development is still unknown. Greater insight in this relationship is clearly crucial when building a true physiological model.

Another assumption of our model is that egg hatch is solely temperature dependent. Our data show that egg hatch is not photoperiod sensitive and no evidence suggests that eggs use chemical cues (L. Holleman, unpublished data). Therefore it seems reasonable to assume that temperature is indeed the only factor affecting egg hatch.

Eggs seemed to develop continuously after laying and did not need a cold period before development can start. This in contrast to some species, such as *Tortrix viridana*, whose eggs will not develop until after a cold period (Du Merle 1999). *Tortrix viridana* diapause is usually broken in late autumn or early winter, which is around the time winter moths lay their eggs. Moreover, experiencing a cold period merely delayed egg hatching, rather than advance it in a similar way as Kimberling and Miller (1988) have reported. Visser and Holleman (2001) correlated egg hatching dates with the number of cold days for the Dutch population of winter moths. Although they did find a correlation, it was a much weaker effect than that found in the Canadian population. Moreover, degree-day models tend to underestimate the development rate at low temperatures. While this cannot explain the effects for the Canadian population, it would be sufficient to explain why Visser and Holleman (2001) found a correlation with the number of cold days. The number of cold days need not be a direct cause of later egg hatch in the Dutch winter moth population.

However, development rate did change over time with egg age. Such a change in development rate needs to be fully understood before incorporation in a model, equally whether it is due to diapause or to some such more gradual change over time; a mean development rate is not sufficient. Rather, it is important either to use different models to describe different separate stages in egg development, or to use a more gradual transition between diapause and rest. Gray (2001) uses a model where gypsy moth development is inhibited by some inhibitory agent which gradually declines as development progresses. Although gypsy moths do have a diapause, both during and after diapause, respiration rate of the eggs increased during development (Gray *et al.* 1995).

The great strength of the physiological model we use here is that its parameters have biological meaning (enzyme activation/inhibition). Incorporating a time or age effect into the model potentially reduces this biological meaning. Egg age might function at the physiological level, for instance by enzyme inhibition reducing the development rate. In

model terms, that would be a change in the denominator. Egg age could also work via some other inhibitory way, in which case this model may not be a good model to use.

Our findings stress the need to understand what happens during development, before we can build a physiological model to predict egg hatching. Development rate can be described very well using a nonlinear curve. Inhomogeneity in development rate over time or egg age is something that needs further study, as it has a large effect on our models predictions.

Acknowledgements
We would like to thank Leonard Holleman, Tanya Jenkins and Jan Nicolay Haye for practical assistance. Baroness Van Boetzelaer Van Oosterhout kindly gave permission to collect winter moth in Oosterhout. The Life Sciences Foundation (ALW grant 812.04.009) supported this research.

Chapter 4: Maternal effects in an insect herbivore as a mechanism to adapt to host plant phenology

Margriet van Asch[1] Riita Julkunen-Tiito[2] and Marcel E. Visser[1]

[1] Netherlands Institute of Ecology (NIOO-KNAW), Centre for Terrestrial Ecology, P.O. Box 40, 6666 ZG Heteren, The Netherlands
[2] University of Joensuu, Department of Biology, Yliopistokatu 7, FIN-80110 Joensuu, Finland

Abstract

Maternal effects may play an important role in shaping the life history of organisms. They can affect offspring size and number, and determine the onset and termination of lifecycle stages. Using an insect herbivore, the winter moth (*Operophtera brumata*) on oak (*Quercus robur*), we show that maternal effects can also shape herbivore phenology in an adaptive manner. Winter moth egg-hatching needs to coincide with oak bud opening, as only freshly emerged leaves are suitable as food. However, there is spatial variation in the timing of bud opening. We show that the generation time between the mother's and her offsprings' hatching dates was shorter for mothers who hatched late relative to bud opening of the tree they fed on, and hence had to feed on older leaves. Furthermore, maternal feeding conditions affected both larval development time of the mother and egg development time of her offspring. We thus show that adaptation to spatial variation may be achieved via maternal effects. While this is a mechanism selected to adapt to spatial variation, it may now also play a role in adaptation to climate change induced shifts in host phenology, and allow insect herbivores to adapt to changes in the timing of food availability without the need for genetic change.

Introduction

In their heterogeneous world organisms need to cope with spatial and temporal variation in environmental conditions. For insect herbivores these conditions are determined by the different plant species they feed on, as these are usually only present or suitable as food during a restricted period of the year. Development outside this period often has severe fitness consequences, even if this is only a few days (Van Asch and Visser 2007). The optimal period may differ between years, as environmental conditions change. In addition to this temporal variation, there is quite often also considerable spatial variation. In deciduous tree species, the individual trees can differ considerably in the timing of bud opening in spring. These differences are predictable, as individual trees are consistently early or late in developing (Crawley and Akhteruzzaman 1988). Insect herbivores may deal with this environmental variation in two ways.

First, insects may adapt locally to their host plant, a process called adaptive deme formation (Van Zandt and Mopper 1998). In order for this to occur, there must to be sufficient genetic variation in herbivore phenology, and selection must be strong enough to counteract the effects of gene flow, resulting from dispersal between populations on individual host plants.

Alternatively, organisms may only vary in their phenotype in response to environmental conditions. In other words, insects may be phenotypically plastic, expressing different phenotypes under different environmental conditions. Such phenotypic plasticity may be adaptive, enabling animals to perform better in spatial or temporal variable environments (Falconer and Mackay 1996). Responses to environmental conditions can change (genetically) under selection, for instance in changing environments. Examples include mosquitoes (Bradshaw and Holzapfel 2001) and several bird species (Pulido and Berthold 2004).

This means that insects need to respond to environmental cues during development. Such cues should be those environmental variables that best predict the environmental conditions the individual will encounter during its life. These can be variables experienced during their own development, but sometimes environmental variables experienced by the parents (most commonly the mother) gives a better prediction of the best phenotype of their offspring. In such cases, maternal effects may play a role: genetic or environmental differences in the maternal generation affect the phenotype of the offspring. Maternal effects are thus a special form of phenotypic plasticity acting across generations (Mousseau and Dingle 1991). Maternal effects are increasingly recognized for their role in adaptation to variable environments (Mousseau and Fox 1998). Known maternal effects include the number and size of offspring (Mousseau and Fox 1998), and the amount of resources invested by the parents (Rossiter 1996). Other examples include the determination of development time in birds via yolk hormones (Gorman and Williams 2005), diapause in insects (Mousseau and Dingle 1991), germination time (Etterson and Galloway 2002) and dormancy in plants (Roach and Wulff 1987). Maternal effects may either decrease or

increase the rate of response to selection and thus accelerate or slow down evolutionary change (Kirkpatrick and Lande 1989).

Phenology plays an important role in many insects feeding in spring; host plants are usually only available for feeding during a limited period of time (Van Asch and Visser 2007). Synchrony with the host plant is crucial as fitness consequences of asynchrony are severe; a five day difference leads already to a 90% drop in survival (e.g., Van Asch et al. 2007). Are maternal effects a mechanism to maintain synchrony with the host plant?

We will study maternal effects on offspring phenology using the winter moth (*Operophtera brumata*), which feeds preferentially on oak (*Quercus robur*) leaves. Only the young oak leaves are suitable for feeding (Feeny 1970; Wint 1983; Tikkanen and Julkunen-Tiitto 2003), so eggs need to hatch within a few days of bud opening of the host tree. There can be large differences (up to three weeks) in the timing of bud opening between individual oak trees, even in adjacent trees. Moreover, these individual differences are highly consistent over time (Crawley and Akhteruzzaman 1988; Van Dongen et al. 1997; M. van Asch, unpublished data). There is some evidence of local (phenotypic) adaptation to individual host trees (Van Dongen et al. 1997), with late hatching eggs on late developing trees. One of the conditions for local adaptation is limited dispersal. Winter moth females are wingless, hence females usually lay eggs on the tree on which they developed. Males do have wings, and although they generally do not disperse far (Van Dongen et al. 1996) they are still more likely to disperse than females. Thus, feeding conditions of the mother are a better predictor of feeding conditions for her offspring than are those of the father, one of the prerequisites for adaptive maternal effects to occur (Donohue 1999; Galloway 2005). In winter moths, maternal effects can therefore potentially play a major role in achieving synchrony with the host tree.

There are two ways in which the timing of the mother can influence the timing of her offspring. The first is through a direct effect of feeding conditions on the larval development time of the mother. If the other life stages are unaffected by leaf age (pupal time of the mother, and egg development time of her offspring) this would then indirectly lead to an effect on egg hatching time of her offspring. Leaf age does indeed affect larval development time (Tikkanen and Lyytikainen-Saarenmaa 2002) as well as growth rate (e.g., Feeny 1968). In addition, there should also be a fixed period between pupation and eclosion of the adults, and between egg laying and egg hatching. There is evidence for the latter; egg laying date and egg hatching date are correlated in the winter moth (M. van Asch, unpublished data), as well as in other species such as the gypsy moth (Ruohomaki et al. 1993). Although the relation between pupation and adult ecclosion could be disrupted by pupal diapause, it has been now been shown that pupal diapause does not exist in the winter moth (Peterson and Nilssen 1998 and references therein). Secondly, feeding conditions of the mother may have an effect directly on the eggs themselves, leading to differences in the development time of her offspring. This can happen, for instance, if a mother can change the amount of resources she puts into her eggs, depending on her own feeding conditions.

Feeding conditions change over time as water and nitrogen content of leaves decrease and leaf toughness and phenolics compounds increase. Winter moths pupate at a lower weight when they feed on older leaves with a higher condensed tannin concentration (Feeny 1968; Tikkanen and Julkunen-Tiitto 2003). Tannins have also an effect in the following generation: sons of gypsy moths (*Lymantria dispar*) reared on red oak (*Quercus rubra*) had a lower pupal weight if their mothers fed on leaves with high condensed tannin concentration (Rossiter 1991), while daughters had a shorter prefeeding period (associated with dispersal tendency). Thus, phenolic compounds not only have a direct effect on the mothers, but also a maternal effect on the offspring.

The aim of this study was to determine whether timing of egg hatch (and thus feeding conditions) of the mother can affect the timing of egg hatch in the next generation by either an effect on larval development time of the parental generation, or a direct maternal effect on egg developmental time. We fed the parental generation differently aged leaves, and then measured larval and pupal development time of the parents, and egg development time of the offspring.

Material and Methods

We fed caterpillars on leaves with a different age. We did this by manipulating egg-hatching date, and then feeding caterpillars that thus hatched at different times, on leaves of the same tree, thereby creating groups of caterpillars (the mothers) that experienced a different timing relative to oak bud opening. Both their own development time and the egg development time of their offspring were then determined.

Origin of winter moths

In order to feed caterpillars differently aged leaves, we needed to create differences in egg hatch in the maternal generation. To achieve this, we caught female winter moths (the 'grandmaternal' generation) on oak trees (*Quercus robur*) using insect traps prior to the experiment (November 2002). Females were caught at seven different locations, to the west of Arnhem (05°48' E, 51°59' N), the Netherlands. These females were used to produce the parental generation. Females were provided with a roll of tissue paper to lay their eggs on and placed in a half-open shed during egg-laying. In order to create differences in the timing of egg hatch, eggs from each female were divided over three different temperature treatments, each mimicking a different year (Visser and Holleman 2001): a 'cold' year (1973), an 'average' year (1983) and a 'warm' year (1998). This ensured that each group of caterpillars had a similar genetic composition, and differences in egg hatch between groups were due to experimental manipulation rather than natural variation in egg hatch. At the start of the rearing experiment 60-65% of the eggs had hatched within each corresponding treatment. We used only broods with a known median egg hatch date at the start of the rearing experiment, to prevent the inclusion of (naturally) early or late hatching caterpillars. One day before the experiment started all previously hatched caterpillars were removed, so that caterpillars used in the experiment were maximally 18 hours old when the experiment

started. This setup enabled us to use newly hatched caterpillars from the same females at different times, i.e. with leaves of a different age in the rearing experiment. There was a five-day difference in egg hatching between each of the different temperature treatments; this means that there were five-day intervals between start of feeding (and thus leaf age) in the experiment.

Design of the rearing experiment

Caterpillars fed on leaves of different ages, by feeding leaves from the same tree to caterpillars with the same parents that differed in egg hatching date due to the temperature treatment of the eggs (table 1). Both their own development time and the egg development time of their offspring were then determined.

At the start of the experiment (23rd of April 2003) a tree was selected whose buds had just opened (tree A). The first group of newly hatched caterpillars (A_0: N=320) was fed on leaves from this tree. Five days later a second group of newly hatched caterpillars (A_5: N=189) were fed leaves of the same tree, that by then had leaves that were five days old. The whole experiment was repeated using another tree (B); this tree opened its buds five days later than the first tree (28th of April). Consequently the first group of caterpillars that fed on leaves from the second tree (B_0: N=174) had hatched at the same time as the second group of caterpillars that fed on leaves of the first tree (A_5). The second group of caterpillars fed leaves from the second tree (B_5: N=180) hatched five days later still (3rd of May). Both trees are growing at the NIOO-KNAW in Heteren, the Netherlands, and are approximately 15 years old.

Originally, the design of the experiment included the effect of starvation (i.e. caterpillars that had hatched five days before the oak buds opened). Few of these females survived (A_{-5} 0 females out of 438 caterpillars and B_{-5} 3 females out of 205 caterpillars) and they are not further considered here.

Table 1: Experimental setup. The experiment was started with eggs that hatched at three different dates with five-day intervals in between (eggs from each date experienced a different temperature regime, resulting in a different hatching date). Caterpillars fed on leaves from two different trees (bud opening date shown between brackets). Letters refer to the tree caterpillars fed on; numbers refer to the age of the leaves (days) at the start of the experiment.

	Tree A (23 April)	**Tree B** (28 April)
Egg hatch 23 April	A_0	
Egg hatch 28 April	A_5	B_0
Egg hatch 3 May		B_5

Rearing of the maternal generation

During rearing caterpillars were fed on progressively maturing leaves. Caterpillars were reared individually in glass vials kept in a half-open shed; so that rearing temperature was similar to the outside temperature experienced by the trees. However, if temperatures rose above 20 °C during the first five days of larval life, the caterpillars were temporarily moved to an air-conditioned room at 20 °C to prevent caterpillars dying because of the high temperatures or dehydration. Leaves were replaced with freshly collected new leaves three times a week. Vials were checked daily for pupating caterpillars. Temperature during rearing was measured every 15 minutes using an HOBO temperature data logger (±0.4 °C) and averaged over each hour to calculate the number of degree-days. After pupation, pupae were weighed and transferred to plastic vials containing moistened vermiculite. The vials were stored in a climate chamber (SANYO Incubator MIR-553) at a constant temperature of 12°C until emergence of the adult moths.

Adult emergence and offspring development

In November, vials were checked daily for emerging adults. After emergence females were immediately mated individually to a male whose mother originated from the same location, and who was fed leaves from the same tree as a caterpillar. Female were never mated with their brothers. Emerging males were kept at 6 °C until used for mating. Females were provided with a roll of tissue paper on which to lay their eggs, which were then kept in the half-open shed. The following spring, egg hatch was scored every two days and median egg hatching date was determined for each the brood of each female.

Analysis of maternal effect on developmental times

Time between egg hatching of the mother (at the start of the experiment) and egg hatching of their offspring was analyzed, as well as the larval and pupal development times of the parents and the egg development time of the offspring. All analyses were done using mixed models (SAS v8), with leaf age and tree as fixed effects and area of origin and sexe as random effects. Analyses of larval and pupal development time were done using all available individuals. Those females that survived and reproduced, may form a non-random subset of the total number of individuals we started with, since only half of the pupae produced adults, and half of these were males. However, larval development times did not differ between surviving and non-surviving individuals, and analyzing the results of larval and pupal development time only for those females that survived and reproduced gave the same results. We therefore show only the results of the full data set here.

Age, toughness and chemical composition of the leaves

Leaf characteristics change as the leaves mature. Moreover, leaf characteristics can vary between trees. In order to follow the maturing process of the leaves we fed the parental generation on, and to check for differences between our replicates, we measured leaf toughness and chemical composition of the leaves. We collected 20 leaves, spread over the

whole tree, twice a week, between 8 am and 10 am. We used ten leaves immediately to measure leaf toughness; the other ten (divided randomly over two samples) were frozen in liquid nitrogen immediately after collection, and stored in the laboratory at -80 °C until they could be freeze-dried and ground. We used leaves thus collected in the first week after bud opening, after two and after four weeks to measure chemical composition, and leaves collected after two, three and four weeks to measure condensed tannins.

We determined leaf toughness using a leaf penetrometer (Feeny 1970), which measures the weight needed to puncture the leaf. To decrease measurement error we measured each leaf twice, then took the mean of these two measurements.

Chemical composition (phenolics) was determined using HPLC (Julkunen-Tiitto and Sorsa 2001). All analyses were done in the Natural Product Research Laboratory, University of Joensuu, Finland. For each extraction, we weighed 5-10 mg of ground leaf material, and we added 0.7 ml methanol (100%). The sample was homogenized using an Ultra-Turrax homogenizer for 20 seconds, left on ice for 15 minutes, homogenized again and centrifuged (3 min. at 13000 rpm), after which the supernatant was removed. The extraction was repeated three more times, but with only 5 minutes on ice. Methanol was evaporated using nitrogen gas, and the dry extract was stored in a fridge. Before HPLC, the sample was again dissolved in 0.6 ml 50%-methanol. We identified fourteen different compounds in the leaves: (+)-catechin, ellagic acid, ellagic acid based hydrolysable tannins, gallic acid, gallic acid based hydrolysable tannins, hyperin, isorhamnetin, an isorhamnetin derivative, kaempferol-3-diacetyl-glucoside, kaempferol-3-glucoside, monogalloylglucose, pentagalloyl glucose & a quercetin derivative, quercetin-3-glucoside & quercetin-3-glucuronide and quercetin-3-rhamnoside.

Condensed tannins were determined using an acid butanol assay (Porter et al. 1985). We weighed 5-10 mg of dried and ground leaf powder, and after adding 1.0 ml methanol (100%), 6 ml acid butanol and 0.2 ml iron reagent (2% ferric ammonium sulfate in 2N HCl), the samples were held in a boiling water bath for 50 minutes. After cooling the absorbance was read using a spectrophotometer at 550 nm. We took two replicates from each sample before the colour reaction, and from each replicate colour absorbance was read twice to reduce measurement error; the mean of those two values is used in the analysis. We used purified oak leaf tannins to quantify the amount of tannins.

Results

Effect of leaf age on development time

Leaf age had a clear effect on the larval development time of the parental generation (fig. 1). Caterpillars fed on older leaves pupated four days earlier than caterpillars fed on young oak leaves ($F_{1,367}$=512.6, p<0.01). Pupal time is the same for caterpillars fed on different leaf ages ($F_{1,140}$=2.64, p=0.1), but males had a shorter development time than females ($F_{1,140}$=8.2, p<0.001).The total (larval plus pupal) development time in the parental

generation is shorter for animals fed on older leaves ($F_{1,140}$=17.6, p<0.01), due to the differences created in the larval period.

Leaf age also had an effect on egg development time of the offspring (fig. 1). Eggs from mothers fed on older leaves had a shorter development time than eggs from mothers fed on younger leaves ($F_{1,60}$=5.43, p=0.02).

The combined effect of leaf age on larval development time of the mother and on the development time of her eggs led to a combined effect of leaf age on total generation time: the time from egg hatch of the mother until egg hatch of her offspring. Generation time on older leaves is shorter ($F_{1,60}$=81.5, p<0.01).

As leaves get older, their toughness increases (fig. 2a). The chemical composition of the leaves may also change as the leaves become older. The only compound to show a consistent increase over time was (+)-catechin (fig. 2b). (+)-catechin was almost absent until the leaves were at least two weeks old, but was present in greater quantities in four week old leaves. (+)-catechin is a precursor to condensed tannins, which were also only present in four week old leaves (fig. 2c).

Effect of tree on development time

Development time also differed between trees (fig 1): caterpillars fed on leaves from tree B pupated after a shorter period than caterpillars fed on leaves from tree A even if they were fed on leaves from the same age ($F_{1,367}$=154.8, p<0.01). Pupal period is also shorter for animals fed on leaves from tree B ($F_{1,140}$=40.4, p<0.01). Offspring development time was the same for both trees ($F_{1,60}$=1.07, p=0.3). Total generation time was about ten days shorter on the later developing tree B ($F_{1,60}$=114.0, p<0.01). Tree did not affect the response to differently aged leaves for either larval, pupal or egg development times separately (interactions nonsignificant). Feeding on older leaves reduced the total generation time on tree A with ten days ($F_{1,36}$=72.2, p<0.01), but on tree B with only five days ($F_{1,21}$=9.0, p<0.01)(interaction leaf age*tree: $F_{1,60}$=6.53, p=0.01). There seems to be overcompensation in the case of tree A, as the original difference in egg hatch between the two groups of caterpillars was only five days, but the generation time of the later hatching caterpillars was ten days less. These caterpillars therefore even hatched five days earlier than the ones fed on young leaves (leaf age: $F_{1,36}$=4.07, p<0.01).

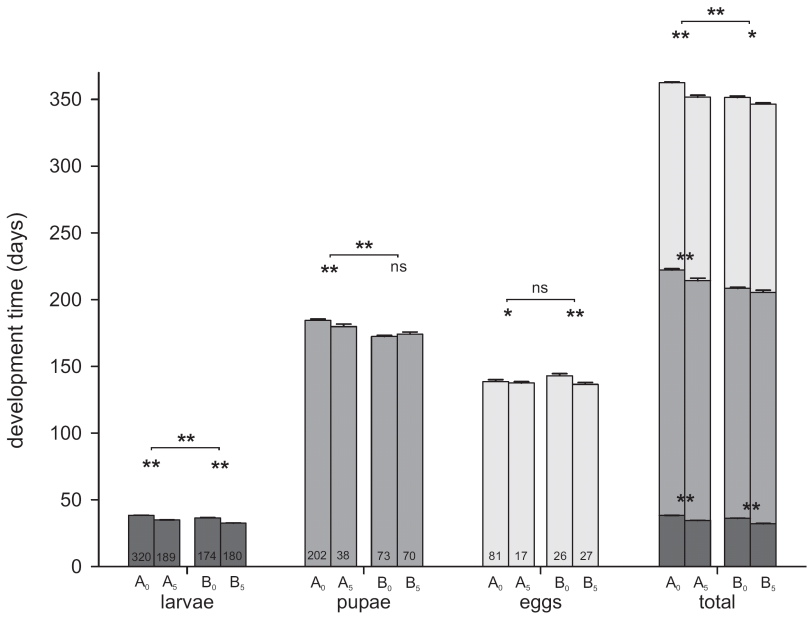


Figure 1 Winter moth development times on differently aged oak leaves. Mean duration of larval (dark grey), pupal (grey) and egg (light gray) stages and the total generation time (days) (± s.e.m.) for caterpillars fed on two different trees (A and B), and on leaves of two different ages (A0 and B0 just opened buds, A5 and B5 five day old leaves at egg hatching). Numbers represent sample sizes, asterisks represent significant differences (**: $p<0.001$, *: $p<0.01$) between leaf ages within tree and between trees.

The increase in leaf toughness is similar in the two trees, and therefore cannot be the reason for any differences in developmental time between caterpillars reared on them. There were no qualitative differences in chemical composition between the two trees; all of the identified compounds (see methods) were present in both trees. Tree A did have more (+)-catechin than tree B at four weeks old ($F_{1,3}=31.2$, $p=0.03$)(fig. 2). Tree A also had a higher concentration of condensed tannins than tree B at four weeks old, but the difference was not significant.

Temperature effects

Clearly, a maternal effect does exist; different rearing conditions of the mother have an effect on timing of egg hatch in the next generation. This could still potentially be due to differences other than leaf age per se. The difference between the trees already suggests that there is at least another additional effect. Different groups of caterpillars hatched with five-day intervals. The environmental conditions (temperature, photoperiod) may have been different for the different groups of caterpillars and the trees they fed on. During the first weeks of rearing mean daily temperatures fluctuated between 12 °C and 16 °C. However, prior to pupation temperatures started to increase (from 26th May onwards). Since caterpillars fed on older leaves hatched later, they experienced a longer period of high temperatures. The shorter development time could therefore be caused by an increased development rate at higher temperature. Expressing the development time in degree-days (DD, calculated by the summing of the mean temperatures per day, above the threshold value) rather than in days can correct for this temperature effect. Using degree-days assumes a linear relationship between development rate and temperature, as well as a certain threshold value below which no development takes place. Degree-days (with a threshold value of 3.9 °C) can describe winter moth egg hatch well (Embree 1970; Visser and Holleman 2001). Larval development time can also be described using degree-days (Topp and Kirsten 1991; Tikkanen and Julkunen-Tiitto 2003). During the larval stage, temperature did not drop below 6 °C, at which temperature there is still development possible. Therefore a threshold value is less critical for our calculations of the larval stage, and we results reported her are based on the same value (3.9 °C) as in the egg stage. However, other threshold temperatures (e.g., 5 °C and 3 °C) give similar results.

Caterpillars fed on old leaves still develop faster than caterpillars feeding on young leaves (leaf age (nested within tree): $F_{2,367}$=25.9, p<0.01), with a difference of 18 DD when feeding on tree A and 25 DD when feeding on tree B. The effect of leaf age on development time cannot be explained by differences in rearing temperature, but is thus truly due to differences in leaf age. The differences between the two trees, however, do largely disappear when expressing development time in degree-days, with a mean difference of only 6 DD between tree A and B (tree: $F_{2,367}$=11.7, p<0.01).

Leaf age also had an effect on development time of the eggs. Since the eggs were kept outside and they were laid at slightly different moments in time, the eggs also experienced slightly different temperatures. Correcting for this by using degree-days still yields the same pattern. Eggs of mothers fed on old leaves developed faster than eggs of mothers fed on young leaves (leaf age (nested within tree): $F_{2,60}$=12.4, p<0.01), with a difference of 40 DD

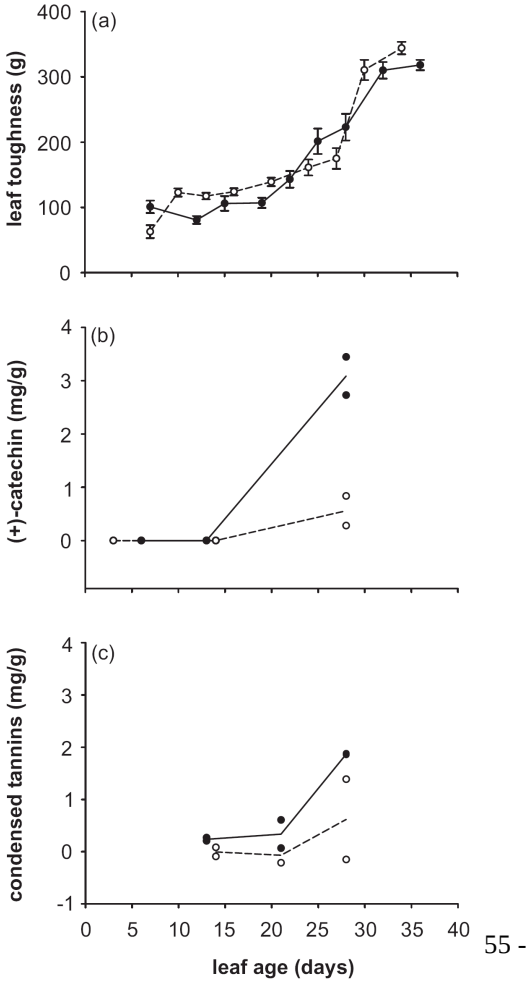


Figure 2 Leaf characteristics in ageing oak leaves. (a) Mean leaf toughness (± s.e.m.) depending on leaf age (days). Leaf toughness is measured as weight (g) needed to puncture the leaf (N=10 in all cases). (b) Condensed tannins (mg/g dry weight) and (c) (+)-catechin (mg/g dry weight) at different leaf ages (days). Lines in (b) and (c) represent mean concentration at each leaf age per tree (n=2 per tree per leaf age).Tree A solid circles, solid line and tree B open symbols, dashed line.

when feeding on tree A and 30 DD when feeding on tree B. Eggs of mothers fed on the later developing tree B had a 15 DD shorter development time than eggs of mothers fed on tree A (tree: $F_{1,60}=20.0$, $p<0.01$).

Another way to circumvent the correlation between our experimental treatment and temperature is to compare larvae fed on the same time but on leaves from trees with a different phenology (i.e., A_5 and B_0). These groups experienced exactly the same temperature and photoperiod. In this comparison caterpillars fed on older leaves also have a shorter development time ($F_{1,\ 106}=40.9$, $p<0.01$). Correcting for temperature differences does not seem to change the effect leaf age has, either on larval development time or on egg development time, but it does reduce the difference between the trees.

Discussion

Synchrony of insect herbivores with the host tree can be maintained through genetic adaptation, and phenology of many insect species, including winter moths (Van Asch et al. 2007), is known to be heritable. We show here that maternal effects can serve as an additional, non-genetic mechanism to become adapted to the phenology of a host tree, as environmental conditions had a clear effect on larval development time of winter moth caterpillars, and thereby on the time until egg hatching of their offspring. Furthermore, if a female hatched after the buds had opened, it took her offspring less time to hatch than when she hatched at the moment of bud opening. This mechanism may thus serve to improve synchrony with bud opening for the next generation, as a shorter generation time for late-hatching parents means that their offspring will be relatively earlier timed than their parents. Selection also acts on this, since feeding on older leaves reduces pupation weight (Feeny 1970; Tikkanen and Julkunen-Tiitto 2003). In the winter moth-oak system a difference of five days reduces pupation weight by a third (Van Asch et al. 2007), and in this experiment females feeding on older leaves had 30 % less offspring. However, the offspring they do have will be better synchronized. For the winter moths, this only works when they are too late, i.e. when feeding on old leaves. Winter moths have a relatively low resistance to starvation, so when they are too early, i.e. hatch before bud opening, this resulted in such strong selection that hardly any females survived to reproduce in the treatment where the eggs hatched five days prior to bud opening. Larva that do manage to survive a short period of starvation have a longer development time than non starved ones (Wint 1983). Due to the strong selection, maternal effects are unlikely to be involved with improving synchrony when hatching too early, and any adaptation will be through genetic change. Some species of moths have a higher resistance to starvation, e.g. gypsy moths (*Lymantria dispar*) (Hunter 1993) and oak leaf roller (*Tortrix viridana*) (Hunter 1990). Maternal effects may play an additional role in determining phenology for those species also when hatching before the food becomes available. Development can only start after food becomes available, and this, possibly in combination with an increase in development

time, leads to the same or a later pupation date in early hatching, starved larvae than in non-starved ones, resulting in later hatching eggs in the next generation.

The factor that is most likely to cause the effect is leaf age. However, photoperiod and temperature are correlated with leaf age, since temperature increased as the season progressed. Analyzing development time in degree-days can for temperature effects to some extent correct. This analysis assumes a developmental threshold temperature, which we know for the egg stage, but less precisely so for the larval stage. However, larval rearing temperatures were relatively high, thus we do know that temperature never dropped below the developmental threshold. The results are relatively insensitive to the threshold value we use; other values produced the same results. Correcting thus for temperature effects reduced the differences between trees, but the effects of leaf age remained unaltered.

Caterpillars that fed at the same time, but on trees with a different phenology, experienced exactly the same environmental conditions and still differed in development time. However, trees differed in their defensive compounds prior to pupation. This could be an effect of temperature, since temperature can reduce both (+)-catechin and tannin levels (Kuokkanen et al. 2004). We would expect earlier pupation and lower pupal weights due to the higher levels of defensive compounds in tree A, but we find the opposite. This again points in the direction of leaf age as the causal factor.

Maybe even more interesting than the direct effect via development time of the mother is the indirect effect of leaf age on development time of the eggs. Both feeding on older leaves and feeding later in the season shortened the development time of the eggs. Feeding conditions are known to have an effect later in life (Rossiter 1991), where offspring weight and dispersal tendency are affected by the amount of phenolics consumed by the mother. We show here that leaf age also affects egg development time. In order to achieve this, there must be something different in the eggs. The mother may vary the amount or the composition of the nutrients she provides the eggs with. In general, larger eggs contain more nutrients. Indeed, it has been shown in gypsy moths that a larger egg size results in faster development time of both eggs and caterpillars (Rossiter 1991) and that feeding conditions of the mother affect the amount of storage protein in eggs (Rossiter et al. 1993). It seems likely that the observed effect works via the amount of nutrients supplied to the eggs by the mother. Feeding on older leaves reduces the pupation weight, and thereby the number of eggs a female lays. Generally, this is assumed to be because females do not have enough resources to lay more eggs. It may, however, also be that those resources they do have are put into fewer eggs, thereby increasing the development rate.

Maternal effects can act as a mechanism to maintain or improve synchrony with the host plant. Although feeding on older leaves led to a decrease in total generation time of only five to ten days, this is similar to the difference we started with. A five-day difference is biologically a very realistic time scale, as this kind of difference in egg hatching can quite easily occur. Moreover, a difference of only a couple of days in leaf age has already marked fitness consequences (e.g., Wint 1983; Tikkanen and Julkunen-Tiitto 2003; Van Asch et al. 2007).

Maternal effects are thought to evolve when the conditions of the mother are a reliable predictor of the conditions her offspring will encounter. In species like the winter moth offspring are more likely to develop on the same tree as the mother than on the tree the father developed on, since females are wingless and thus cannot disperse far. However, if a female does lay her eggs on another nearby tree with a different phenology, then her offspring will end up on a tree whose phenology does not match with their own. Maternal effect may then serve to restore the synchrony in the next generation. Maternal effects serve primarily to deal with spatial variation, as this is highly consistent over time and thus very predictable. However, under changing environmental conditions synchrony with the host plant can become disrupted (Bale et al. 2002; Stenseth and Mysterud 2002; Visser and Both 2005). Then maternal effects provide an alternative mechanism to restore synchronization with the host plant. When the shift in timing is towards starvation due to emergence prior to the availability of food, it may have an effect, but the effect of selection due to increased mortality is then considerable. Especially when the shift in timing is such that it means feeding on older, lower quality food, this can serve as an additional mechanism to adapt to a changing world.

Acknowledgements

We would like to thank Kate Lessells and Erik Postma for helpful comments on the manuscript, and the people from Natural Product Research Laboratory, University of Joensuu for practical assistance with the HPLC-analyses. Baroness Van Boetzelaer Van Oosterhout, ‘Stichting Het Gelders Landschap’, ‘Stichting Het Utrechts Landschap’, the State Forestry Service, the City Council of Renkum, ‘Natuurmonumenten’ and the board of ‘Nationaal Park de Hoge Veluwe’ kindly gave permission to work in their woodlands. The Life Sciences Foundation (ALW grant 812.04.009) supported this research.

Chapter 5: Predicting adaptation of phenology in response to climate change, an insect herbivore example

Margriet van Asch[1], Peter H. van Tienderen[2], Leonard J.M. Holleman[1] and Marcel E. Visser[1]

[1] Netherlands Institute of Ecology (NIOO-KNAW), Centre for Terrestrial Ecology, P.O. Box 40, 6666 ZG Heteren, The Netherlands
[2] Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands

Accepted for *Global Change Biology*

Abstract

Climate change has led to an advance in phenology in many species. Synchrony in phenology between different species within a food chain may be disrupted if an increase in temperature affects the phenology of the different species differently, as is the case in the winter moth egg hatch – oak bud burst system. *Operophtera brumata* (winter moth) egg hatch date has advanced more than *Quercus robur* (pedunculate oak) bud burst date over the past two decades. Disrupted synchrony will lead to selection, and a response in phenology to this selection may lead to species genetically adapting to their changing environment. However, a prerequisite for such genetic change is that there is sufficient genetic variation and severe enough fitness consequences. So far, examples of observed genetic change have been few. Using a half-sib design, we demonstrate here that *O. brumata* egg hatching reaction norm is heritable, and that genetic variation exists. Fitness consequences of even a few days difference between egg hatch and tree bud opening are severe, as we experimentally determined. Estimates of genetic variation and of fitness were then combined with a climate scenario to predict the rate and the amount of change in the eggs' response to temperature. We predict a rapid response to selection, leading to a restoration of synchrony of egg hatch with *Q. robur* bud opening. This study shows that in this case there is a clear potential to adapt - rapidly - to environmental change. The current observed asynchrony is therefore not due to a lack of genetic variation and at present it is unclear what is constraining *O. brumata* to adapt. This kind of model may be particularly useful in gaining insight in the predicted amount and rate of change due to environmental changes, given a certain genetic variation and selection pressure.

Introduction

Climate is changing and will continue to change: global predictions for the next century range from an increase of 1.5 °C to 5.5 °C (IPCC 2001) while on a local scale these changes may be considerably larger. In addition, climate changes may not be uniformly distributed across the whole year. In the Netherlands, for instance, average spring temperature has increased by about 1.5 °C in the last thirty years alone (Verbeek 2003).

Several studies have described the effects of climate change on species (see for an overview (Bale et al. 2002; Parmesan and Yohe 2003; Root et al. 2003) including range shifts and advances of phenology in spring. For instance, trees start leafing earlier (Kramer 1995; Menzel 2000; Root et al. 2003); plants flower earlier in the season (Root et al. 2003) and many birds start breeding earlier (Root et al. 2003). There is however no *a priori* reason why different species within a food chain would shift their phenology at the same rate (Visser and Holleman 2001; Visser et al. 2004). Relatively small effects of climate change can have profound consequences for the ecosystem if species that depend on one another, such as a herbivore and its host plant, react differently to an increase in temperature, thus leading to asynchrony or a mismatch (Visser and Holleman 2001; Bale et al. 2002; Stenseth and Mysterud 2002; Visser et al. 2004).

Climate change induced asynchrony occurs in the interaction between *Quercus robur* (pedunculate oak) and *Operophtera brumata* (winter moth). *O. brumata* eggs hatch early in spring and the caterpillars then feed on young *Q. robur* leaves. Both egg hatch and opening of the tree buds have advanced over the last 25 years (Visser and Holleman 2001). However, egg hatch has advanced much more than tree bud burst, leading to a decrease in synchrony between *Q. robur* and *O. brumata* from a few days to almost two weeks (Visser and Holleman 2001); but see (Buse and Good 1996). If the eggs hatch before the buds are open, there is no food for the caterpillars, and they will starve to death. Moreover, passerine birds such as the great tit (*Parus major*) feed *O. brumata* caterpillars to their young and thus rely on the synchrony between egg hatch and oak bud burst. P. major has not advanced its egg laying to match the earlier caterpillar peak in the Netherlands (Visser et al. 1998), another example of climate change induced asynchrony.

Climate change disturbs interactions between species, and in the case of *O. brumata* asynchrony occurs because their phenotypic response to temperature is too strong. However, phenotypic responses can change if there is heritable variation in the underlying genetic basis determining phenotypic plasticity. The unresolved question is at what rate these evolutionary changes will take place and whether natural selection will lead to a restoration of synchrony. Many studies have reported phenological changes due to climate change, but only a few have reported on the genetic basis of these changes. One study (Bradshaw and Holzapfel 2001) reported a shift in photoperiodic response in a mosquito (*Wyeomyia smithii*), another study reported an advance in timing of breeding in a red squirrel (*Tamiasciurus hudsonicus*)(Réale et al. 2003). In both studies, these changes occurred within a few generations, but long-term predictions were not made. Even if

sufficient genetic variation exists, genetic correlations between traits may limit a response, as seems to be the case in for instance a prairie plant (*Chamaecrista fasciculata*)(Etterson and Shaw 2001).

We investigate the (a)synchrony between *O. brumata* egg hatch and *Q. robur* bud burst, asking whether *O. brumata* will be able to adjust its egg hatch to the changing timing of *Q. robur* bud burst. We start by testing whether *O. brumata* egg hatching is heritable, and we estimate how strong selection intensity due to asynchrony is. We then build a model to predict the response to selection. This requires combining four pieces of information: (i) future changes in spring temperatures, (ii) the advance of *Q. robur* bud burst, (iii) the amount of genetic variation in egg hatch in response to temperature, and (iv) the intensity of selection due to asynchrony between egg hatch and bud burst. For the first two aspects, we rely on existing models for climate change (Esch 2005) and bud burst (Kramer 1994), respectively. We add new data on the genetic variation in the reaction norm (Scheiner 1993) of *O. brumata* egg hatching in response to temperature, as well as the consequences of asynchrony for the fitness of the moths. We then build a selection model for the genetic change in egg hatching reaction norm with increasing temperatures. This enables us to predict the response to selection, given the ambient environmental conditions, in a focal year. We are thus able to predict at what rate *O. brumata* may adapt to its changing environment, and determine whether this rate can match the rate at which climatic change will alter their environment, i.e. the bud burst date of *Q. robur*.

Material and Methods

General setup half-sib experiment

We performed a half-sib experiment in order to investigate whether the strength of the effect of temperature on *O. brumata* egg hatch (the reaction norm) is heritable, and to estimate the amount of genetic variation in the intercept and slope of the reaction norm.

Prior to the experiment, the parental generation was reared in the lab. This ensured that all individuals had experienced similar rearing conditions. Thus, differences between their offspring were not due to differences in rearing of the parental generation.

Trios were formed in the parental generation: one male was always mated to two females. Thus, we created groups of eggs (their offspring) with different degrees of relatedness, and thus of genetic similarity: full sibs, half sibs and unrelated offspring. These eggs were used in the experiment.

In the experiment, the eggs from each female were divided over three temperature treatments. Within each temperature treatment, the eggs were divided over several replicas. Median egg hatching in each replica from each female in each temperature treatment was determined.

This yielded information not only on the phenotypic variation within and between treatments, but also on which part of this variation was due to genetic variation, since the eggs had different degrees of relatedness (Falconer and Mackay 1996).

Origin and rearing of the parental generation in the half-sib experiment
Prior to the experiment (November 2000) we caught female *O. brumata* on *Q. robur* trees. Mated females were caught in four different forests in the Netherlands, all in the area around Arnhem: Oosterhout (05°50' E, 51°55' N), Doorwerth (05°48' E, 51°59' N), Warnsborn (05°50' E, 52°05' N) and Goffert (5°50' E, 51°49'N). Females were kept individually in plastic containers and were provided with a roll of tissue paper to lay their eggs on. These eggs were kept in an outdoor insectarium until egg hatch. Hatching was checked three times a week, and at the median egg hatching date 10 caterpillars per brood were collected (April 2001). These caterpillars were reared individually on fresh oak leaves in small vials. After pupation, pupae were stored individually in plastic vials containing moistened vermiculite. These vials were kept at a constant temperature of 12˚C in a climate cabinet (SANYO Incubator MIR-553) until emergence of the adult moths (November 2001). After emergence, all individuals were kept at 5 ˚C until all adults had emerged. At that time, the adults were used to setup a mating structure (see below). From each originally caught female only one offspring was included in the parental generation of the experiment.

Mating structure in the parental generation of the half-sib experiment
The mating structure was not set up until all males and females of the parental generation had emerged to prevent assortative mating based on adult ecclosure date. In the experiment each male (n=10) was mated to two different females (n=20). Matings were all made within forest of origin (Doorwerth: n=2, Goffert: n=1, Oosterhout: n=5 or Warnsborn: n=2). Females were kept individually in plastic containers. Males rotated every two days between the two females. Females were provided with a roll of tissue paper to lay their eggs on. During mating and egg laying animals were kept in the outdoor insectarium. The eggs that were laid by each of these females were the eggs that we used in the half-sib experiment.

Temperature treatment of offspring in half-sib experiment
The eggs that were laid by the females in the parental generation were divided in either six or nine batches per female. This depended on the number of eggs that were laid: each batch contained at least fifteen eggs. Batches of eggs were divided over three different temperature treatments, with two or three replicates per female per temperature treatment.

Rather than using constant artificial temperatures in each treatment, we used fluctuating temperatures mimicking specific years (Visser and Holleman 2001). These provided a much more natural temperature pattern than using artificial constant temperatures would have done. One of our temperature treatments mimicked a relatively cold year with an average temperature from January 1st until April 1st of 3.5 °C. (1986). The two other treatments mimicked two warmer years (1988 and 1998) with almost the same

average temperature (4.5 and 4.6 °C. from January 1st until April 1st, respectively) but with different temperature patterns over time. The climate pattern in these years was mimicked in climate cabinets (SANYO Incubator MIR-553) using three phases a day: 6 hours at the daily minimum temperature, 12 hours at the mean of the daily maximum and daily average temperature and 6 hours at the daily average temperature. These temperatures were changed three times a week according to the temperatures in the mimicked year (Visser and Holleman 2001). Eggs were checked three times a week for hatching (April 2002). For each batch of eggs from each female median egg hatching date was determined.

Statistical analysis half-sib experiment

Data were analyzed using a General Linear Model (SAS v8). The dependent variable was the temperature sum (above 3.9 °C) from January 1st until median egg hatch. Temperature sum is thought to reflect the mechanism that determines egg hatch (Embree 1970). Fluctuations in temperature during egg hatching can also be corrected using temperature sum. Summing of the daily temperatures from January 1st until median egg hatch, with a threshold value of 3.9 °C, gives the temperature sum. In the sire component offspring of both females (dams) mated to the same male (sire) are included. Dams were nested within sires. Sire and dam components were tested as random factors. In all replicates more than ten eggs hatched. Variance and covariance estimates were obtained using a mixed procedure (SAS v8).

General setup fitness experiment

The consequences of asynchrony for the fitness of *O. brumata* were determined experimentally. We then used these fitness estimates in the predictive model (see below). We used these estimations rather than existing data on fitness effects, since our fitness measurements were done using the same populations as were used for the half-sib experiment.

Caterpillars were reared individually on leaves of different age and/or experienced a starvation period of several days before feeding started. This was done by feeding caterpillars on leaves of different trees with a difference in bud burst date and thus in leave age. Caterpillar survival and pupation weight, which correlates with fecundity (Buse et al. 1998), were measured as indicators of fitness.

Origin of the caterpillars in fitness experiment

Prior to the fitness experiment (November 2002) female *O. brumata* were caught on mature *Q. robur* trees. Females were caught in the same forests as in the half-sib experiment (see above) and in Rhenen (05°35’ E, 51°58’ N). Females were kept individually in plastic containers and were provided with a roll of tissue paper to lay their eggs on. After hatching, caterpillars from these eggs were used in the fitness experiment. The fitness experiment was repeated twice with an interval of five days. To achieve a five-day difference in egg hatching date, the eggs of each female were divided over two different temperature

treatments (SANYO Incubator MIR-553), each mimicking a specific year (Visser and Holleman 2001)(see above): a 'colder' year (1983) and a 'warmer' year (1998). This setup enabled us to use offspring from the same females in both replicas of the experiment that started on different days. One day before the experiment started all previously hatched caterpillars were removed, so that caterpillars were maximally 18 hours old at the start of the experiment. Only broods with a median egg hatch around the start of the experiment were used.

Rearing conditions in fitness experiment

Groups of caterpillars were fed leaves from different *Q. robur* trees and of different leaf ages: one tree had opened its buds five days prior to the start of the experiment, one tree opened its buds at the start of the experiment and one tree opened its buds five days after the start of the experiment. Two different series were started with a five-day interval in between. All trees used were standing together at the NIOO-KNAW in Heteren, The Netherlands, and were approximately 15 years old.

Caterpillars were reared individually in glass vials. The caterpillars that were starved for five days were kept inside in a climate cabinet (SANYO Incubator MIR-553) at a temperature pattern mimicking an 'average' year (1983). Average temperature during starvation was 12 °C. The other caterpillars were all placed in an outdoor insectarium; ensuring rearing temperature was similar to the outside temperature, as experienced by the trees. However, if temperatures rose above 20 °C in the first five days after feeding started, caterpillars were moved temporarily to an air-conditioned room at 20 °C in order to prevent caterpillars dying because of the high temperatures and/or dehydration. As soon as temperatures dropped below 20 °C, caterpillars were again moved to the insectarium. Leaves were replaced with freshly collected new leaves three times a week. Vials were checked daily for pupating caterpillars. Pupae were weighed and larval survival was determined.

Statistical analysis fitness experiment

Larval survival was analyzed using a Generalized Nonlinear Model (SAS v8). Pupation weight was analyzed using a General Linear Model (SAS v8). In both cases, both leaf age and series were included in the model.

To obtain a relative fitness curve survival was expressed relative to the survival of the larvae fed leaves of age 0 at the start of the experiment. Pupation weight was also expressed relative to pupation weight of the larvae fed leaves of age 0 at the start of the experiment. Multiplying these values then gives a relative fitness value. A Gaussian curve was then fitted with relative fitness as dependent variable and age of the leaves at egg hatch as independent variable. Age of the leaves is given as temperature sum (with a threshold value of 3.9 °C) rather than in days. This enabled us to estimate fitness in different years, with different temperature regimes, using our experimentally determined fitness curve. Calculating the fitness per female instead of per egg then corrects for the variation in egg

hatching within a brood (as obtained from the within treatment replica's in the half sib experiment). The width of this fitness curve (relative fitness against timing of median egg hatch relative to oak bud opening, expressed as temperature sum) can then be used as an estimate of the strength of selection in a simulation model.

The simulation model

In order to predict changes in winter moth egg hatching, we developed a simulation model. In this model the response (R) to selection is determined by the heritability (h^2) (e.g. how much do offspring genetically resemble their parents) and by the selection (S) (e.g. which part of the population survives, and thus passes on their genes to the next generation) (Falconer and Mackay 1996): $R=h^2*S$.

Egg hatching is dependent on environmental cues, which will differ between years. This means that the response to selection can differ between years, depending on the conditions in a given year. We therefore used a multivariate version of the equation (Van Tienderen and Koelewijn 1994): this gives the response across all possible environments in the next year to the selection in a given environment (x_i) in year *i*. Since the setup of the half-sib experiment was such that in effect there were only two different temperature treatments (see above), we used a linear reaction norm to describe egg hatch. Both the intercept and the slope of the reaction norm can potentially change through selection. The change in the parameters of the reaction norm (Δ_{gmean}) in a given year (*i*) was calculated by multiplying a generalized genetic covariance matrix (G_g) with a vector $(1 \quad x_i)^t$ characterising the environment (x_i) and with the selection gradient (β) in this year (Van Tienderen and Koelewijn 1994): $\Delta_{gmean} = G_g (1 \quad x_i)^t \beta$.

The selection gradient (β) depends on the mean trait value (z_{mean}) relative to the optimal population mean (z_{opt}) for a trait z. It also depends on the variation in the trait (zs) as well as on the strength of selection (ws) (Van Tienderen and Koelewijn 1994):
$\beta = -(z_{mean} - z_{opt})/(ws^2+zs^2)$.

We can then calculate the expected change in reaction norm in year *i* due to selection. This gives the new reaction norm, so that the process can be repeated, with new environmental x_{i+1} values and a new selection gradient, depending on the conditions in year *i+1*.

Estimation of the model parameters

Both the mean reaction norm at the beginning of the simulation and the phenotypic variation in egg hatching (zs), as well as the generalized genetic covariance matrix (G_g) were derived from the data from the half-sib experiment. In the half-sib experiment, we created a set of specific temperature treatments. Thus, we know the variance and covariance only for these specific temperatures. In the model we used a more general description, the generalized genetic covariance matrix (G_g), allowing for interpolation to other temperature values. This generalized matrix (G_g) can be calculated from the specific covariance matrix

following Van Tienderen and Koelewijn (1994). The width of the fitness curve (as determined from our fitness measures) determines the strength of selection (ws).

Two pieces of information complete the model. The environment in each year (x_i) was characterised by the average temperature from January 1st until April 1st, as predicted from a climate model. We used an IPCC-SRES model, with an intermediate increase in temperature (SRES-B2) (Esch 2005). This climate model predicts the daily mean and maximum temperatures until 2100 for a specific region (50*50 km), in this case the area around Arnhem, the Netherlands. The optimal moment of egg hatch (z_{opt}) depends on the moment of *Q. robur* bud burst. We used Kramer's well-established sequential model (Kramer 1994) to predict bud burst. This model provides a good description of *Q. robur* bud burst in the Netherlands (Visser and Holleman 2001).

Results

Half-sib experiment

Temperature had a major effect on egg hatch ($F_{2,8.7} = 211.7$; $p < 0.001$) (see fig. 1): in the treatments with a higher temperature eggs hatched at a higher temperature sum (even though this temperature sum is reached about two weeks earlier). Offspring of different females react differently to the different temperature treatments (interaction dams (nested within sire) * treatment: $F_{20,8.7} = 1.92$; $p = 0.02$). These differences can be due to both additive genetic variance and maternal effects (Falconer and Mackay 1996). However, offspring of different males also reacted differently to the temperature treatments (interaction sire * treatment: $F_{18,20.2} = 2.59$; $p = 0.02$), indicating that these differences are caused by additive genetic variance (Falconer and Mackay 1996). We can conclude from this analysis that the slope of egg hatching reaction norm is heritable. Heritabilities within treatments, estimated as additive genetic variance divided by phenotypic (total) variance (Falconer and Mackay 1996), are quite high ($h^2 = 0.80$, $h^2=0.63$ and $h^2=0.94$), as are the estimated additive genetic correlations between the treatments (0.87, 0.91 and 1).

Fitness

Both leaf age (df=2, Wald Stat.=229.5, p<0.001) and series (df=1, Wald Stat.=5.09, p=0.02) had an effect on larval survival (see fig. 2a). Starvation greatly increased mortality: after five days of starvation more than 90 % of the caterpillars died, whereas only 40 % of the non-starved caterpillars died (df=1, Wald Stat.=247.1, p<0.001). Feeding on older leaves did not

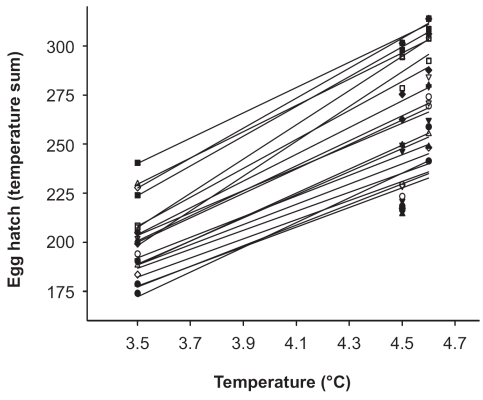


Figure 1 Egg hatching reaction norms in the half sib experiment. Egg hatching is represented as temperature sum (above 3.9 °C) from January 1st until median egg hatch. Treatments are

represented as the average temperature from January 1st until April 1st. Each line represents the mean reaction norm of the progeny of one female. A reaction norm is obtained by fitting a regression line (least squares methods) through the data points of a female. Each symbol within treatment represents the temperature sum at median egg hatching of a female, averaged over the replicates within treatment. Two females mated to the same male are represented by the same symbols.

have an effect on survival (df=1, Wald Stat.=0.10, p=0.75). Larval survival decreased by about 30 % as the season progressed. However, the difference between the series was not the same for the different leaf ages (leaf age * series: df=2, Wald Stat.=22.27, p<0.001). Survival in the second series was lower than in the first series for both caterpillars fed on young leaves and for caterpillars fed on older leaves (df=1, Wald Stat.=20.91, p<0.001 and df=1, Wald Stat.11.7, p<0.001, respectively), whereas for the starved caterpillars survival was slightly higher in the second series than in the first series (df=1, Wald Stat.=5.88, p=0.02).

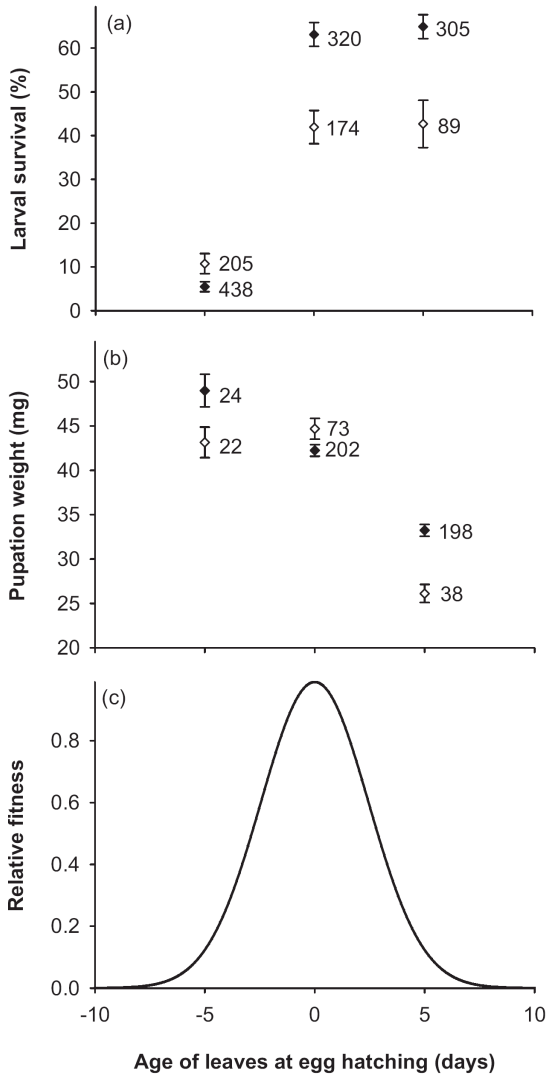


Figure 2 Larval survival (a), pupation weight (b) and relative fitness (c) of caterpillars fed on leaves of different ages. Data points (a and b) represent means (±

S.E.M.) for the different series (first series closed symbols, second series open symbols). Numbers denote sample sizes. Relative fitness (c) is the fitness relative to the treatment with the highest fitness and is calculated from combining both survival and weight estimates.

The effect of leaf age on pupation weight differed between the different series (leaf age*series: $F_{1,551}$=12.6, p<0.001) (see fig.2b). Both starved caterpillars ($F_{1,44}$=5.3, p=0.03) and caterpillars fed on older leaves ($F_{1,234}$=19.6, p<0.001) had a higher pupation weight in the first series, while caterpillars fed on leaves that had just opened did not differ in pupation weight between the different series ($F_{1,273}$=3.6, p=0.06). Caterpillars fed on older leaves had a lower pupation weight (first series: $F_{1,398}$=92.4, p<0.001, second series: $F_{1,109}$=107.7, p<0.001), while starved caterpillars in the first series even had slightly higher pupation weights ($F_{1,224}$=11.5, p<0.001) compared to the larvae fed on young leaves.

Hence, caterpillar fitness depends on synchrony of egg hatch with oak bud burst: caterpillars hatched after bud burst have to feed on older leaves, which leads to a reduction in pupal weight and thus in fecundity, while starvation clearly leads to a decrease in survival. Fitness values closely resemble those reported previously (Feeny 1970; Wint 1983; Tikkanen and Julkunen-Tiitto 2003). These fitness values were then used to calculate a relative fitness curve (see fig. 2c). This curve is then used in the simulation model.

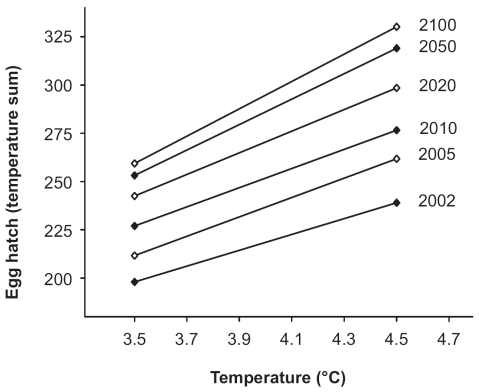


Figure 3 Predicted changes in mean egg hatching reaction norm across an environmental range for the next century. Egg hatching is represented as temperature sum (above 3.9 °C) from January 1st until median egg hatch. Environments are represented as the average temperature from January 1st until April 1st. Each line represents the mean reaction norm in a year.

Simulation model

Temperature in winter and early spring will increase in the Netherlands by 3.5 °C in the next century under the climate model (Esch 2005) we used. Due to the increase in temperature, oak bud burst will further advance by about eight days ($F_{1,97}$ = 12.9; p < 0.001) (see fig. 4). As a reference, we predicted the change in egg hatching date in the absence of any response to selection, so that any change was entirely due to the current phenotypic plasticity (see fig. 3 and 4). Under these circumstances egg hatch will advance two weeks

($F_{1,97}$ = 53.5; p < 0.001). Thus, egg hatch will advance more than oak bud burst, leading to an increasing difference between egg hatch and oak bud burst (from two weeks now to three weeks by the end of the century; timing difference (days): $F_{1,97}$ = 16.8; p < 0.001). Our findings suggest that in the absence of genetic change synchrony will be disrupted even more.

The model was then run including a potential response to selection. This had a large effect on the predicted synchrony between egg hatch and bud burst. Mean egg hatching reaction norm changed (fig. 3), resulting in a much closer match of egg hatching with oak bud burst (fig. 2). This is due mainly to a change in the intercept, not in slope, of the reaction norm: in future, eggs will need more warmth before they hatch. Due to selection, even within a few years the difference between egg hatch and oak bud burst is predicted to be much smaller than it would be without an evolutionary change in reaction norm. After 20-30 years, the model predicts that synchrony will be completely restored, even though the asynchrony in some individual years will still be quite large.

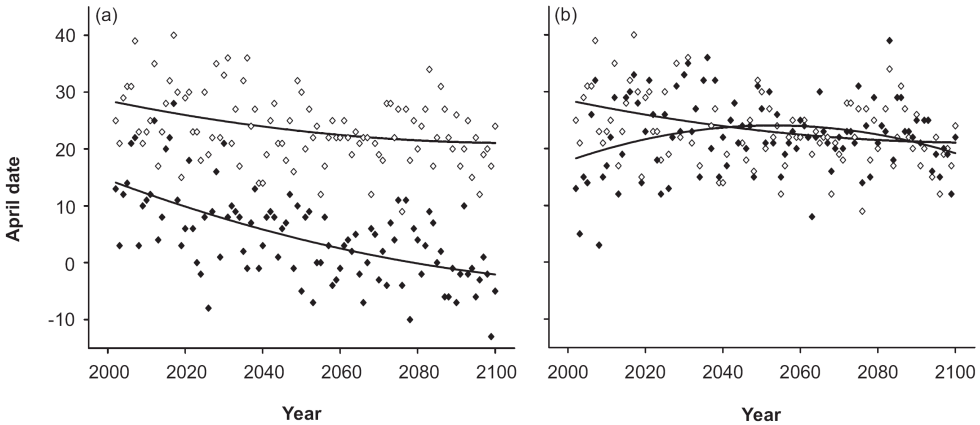


Figure 4 Predicted *O. brumata* egg hatching date and *Q. robur* bud burst date (all dates in April dates, i.e. day 1 = 1st of April, day 31 = 1st of May) for the next century. Bud burst date (open symbols, a and b) is predicted using Kramer's sequential model (Kramer 1995). Egg hatch (closed symbols) is predicted from the model, both without a response to selection (a) and including the predicted response to selection (b). Temperatures used in these models are the temperatures as predicted by the climate model (Esch 2005).

Discussion

Climate change leads to an advance in phenology in spring in many species. Synchrony between different components of a food chain may decrease if an increase in temperature affects phenology of different species differently, as is the case in the winter moth egg hatch – oak bud burst system. We demonstrate here that egg hatching reaction norm is

heritable, and that sufficient genetic variation exists to adapt to the phenology of the oak trees. Coupled with the severe fitness consequences of even a few days difference between egg hatch and oak bud opening, we predict a rapid response to selection, leading to a restoration of synchrony of egg hatch with oak bud opening. This is an example were a species has a clear potential to adapt genetically to their changing environment.

However, the predicted rate of adaptation by the model is at variance with the current observed asynchrony of the winter moth (Visser and Holleman 2001). Apparently, the rate of adaptation is lower than we predict. Several factors can potentially hamper the response to selection.

An assumption of the model is that variance remains constant during simulation, while it may decrease after several years of selection. This is unlikely to cause any problems, since the predicted change in reaction norm is already within the range of reaction norms we see in the half sib experiment. Population size is also not taken into account. Populations cannot go extinct in the model, while in reality they can. However, females have a large reproductive capacity; they can lay up to 300 eggs. Even a few individuals are sufficient to restore population size within a few generations.

Another factor may be an overestimation of genetic variances in the half-sib experiment. Winter moths were caught in four different areas. Due to low densities of winter moth previous to the experiment coupled with a large mortality in the parental generation sample sizes were too small to reliably estimate genetic variances within any one of the forests separately. Even though the forests are all in the same general area within the Netherlands, dispersal between the areas is probably very limited, since females are wingless and males generally do not disperse far (Van Dongen et al. 1996). However, phenotypic variation within populations is still considerable (M. van Asch, unpublished data), making it highly unlikely that this is the only explanation for the observed difference. The actual rate of adaptation may be slightly lower than what we expect based on our estimations, but even so we would expect the general picture to remain unaltered.

The difference between the observed asynchrony and our expected rapid adaptation must then come have another cause. Our fitness estimations are based on the assumption that the optimal moment of egg hatch is determined by the moment of *Q. robur* bud burst. *O. brumata* is not a specialist species: it can also feed on leaves from tree species other than oak (Watt and McFarlane 1991; Kerslake et al. 1998; Tikkanen et al. 2000). All our study areas consist of predominantly *Q. robur* (European oak). However, several other tree species, such as (*Quercus rubra* (red oak), *Acer pseudoplatanus* (maple) and *Betula pendula* (birch) are also present. In Oosterhout, for instance, the vast majority of the mature trees are *Q. robur* trees, but with a few immature *Q. rubra* and *A. pseudoplatanus* trees underneath. This means that selection pressures acting on *O. brumata* to hatch later are actually less severe, since most other tree species present open their buds earlier than *Q. robur*.

A long-term possibility to adapt to climate change may then also include systematic host shifting to a tree species with a more suitable bud burst. Only one of our study areas

consists of only mature *Q. robur* trees and has no undergrowth at all. Rather interestingly, population size in this area has been consistently low for the past several years, hinting at a stronger selection in this population than in the other study areas. Moreover, we have some indications that the timing difference here may have become smaller in the past several years, whereas it has not done so in the other study areas. This is certainly something that needs further investigation, by combining field data on synchrony with experiments to determine whether the eggs' response to temperature changes over time and between populations.

If the differences between populations in oak and mixed forests hold true it means that *O. brumata* has two options available to respond to a change in climate. If there are other tree species present, they may shift to this other tree species. In the absence of another host species, winter moths will respond to selection, which will then lead to a restoration of synchrony with *Q. robur* bud burst within a few decades. A similar rapid adaptation is observed in, for instance, *W. smithii* (Bradshaw and Holzapfel 2001). This illustrates that at least some species may be able to change genetically and thus adapt to the environmental changes induced by climate change. This kind of model may be particularly useful in predicting the effects of environmental changes, to gain insight in the expected amount and rate of change, given a certain genetic variation and selection pressures. However, a certain amount of caution is necessary, since even if species have a clear potential to adapt to climate change, as in the case of *O. brumata*, there may still be other factors hampering a rapid response to selection.

Acknowledgments

We would like to thank Hans Luthardt and Monika Esch (Max Planck Institute for Meteorology) for providing the temperature data from the climate predictions. Kate Lessells, Erik Postma, Arie van Noordwijk and two anonymous referees provided helpful comments on an earlier version of the manuscript. Baroness Van Boetzelaer Van Oosterhout (Oosterhout), 'Stichting Het Gelders Landschap' (Warnsborn), 'Stichting Het Utrechts Landschap' (Rhenen), the State Forestry Service (Doorwerth), the City Council of Renkum (Doorwerth) and the board of 'Nationaal Park de Hoge Veluwe' kindly gave permission to work in their woodlands. The Life Sciences Foundation (ALW grant 812.04.009) supported this research.

Chapter 6: Climate change induced genetic shifts in an insect herbivore's temperature sensitivity

Margriet van Asch, Leonard J.M. Holleman and Marcel E. Visser

Netherlands Institute of Ecology (NIOO-KNAW), Centre for Terrestrial Ecology, P.O. Box 40, 6666 ZG Heteren, The Netherlands

Abstract

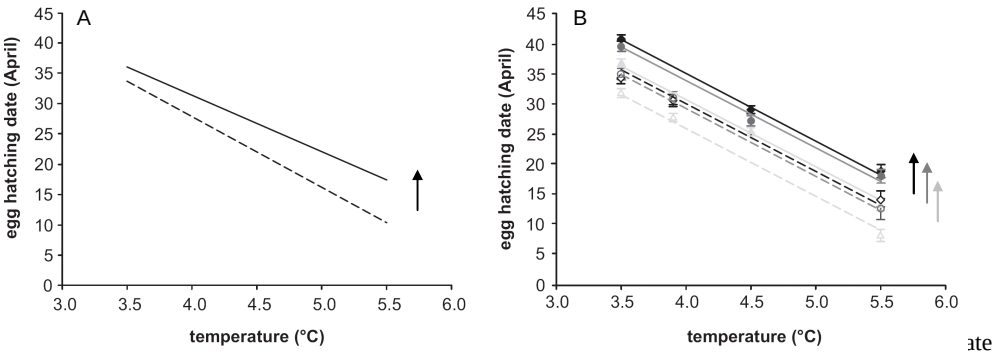
Under changing climatic conditions, species need to adapt to their new environment, but examples of climate-induced genetic changes in wild populations have been few. Using both long-term observational data and experiments, we show that temperature sensitivity has changed genetically in an insect herbivore (*Operophtera brumata*), resulting in closer synchrony with the phenology of its food plant (*Quercus robur*). The observed rate of change matches the predicted rate of change of one day per year. Hence, altered selection pressures, caused by environmental change, result in a rapid adaptive response in temperature sensitivity of insect phenology.

Introduction, results and discussion

Many organisms currently face rapidly changing environmental conditions, due to anthropogenic changes in climate or land use (Bale et al. 2002; Stenseth et al. 2002; Parmesan and Yohe 2003; Root et al. 2003; Bradshaw and Holzapfel 2006). A major challenge is to predict the rate at which populations will be able to adapt. If this rate lags behind the rate at which the environment changes, this will have major consequences for population viability (Both et al. 2006; Bradshaw and Holzapfel 2006). One way of coping with changes in the environment is phenotypic plasticity - that is, the ability of one individual (genotype) to express different phenotypes depending upon the environmental conditions (Pigliucci 2001). Phenotypic responses to changes in climate are relatively often reported, and include changes in phenology, i.e., the natural timing of events, such as plant leafing (Kramer 1995; Menzel 2000; Root et al. 2003), bird egg laying and migration (Root et al. 2003) and butterfly emergence (Roy and Sparks 2000) all advancing in the season. However, phenotypic changes are often not sufficient (Nussey et al. 2005; Visser and Both 2005). If species are to adapt to a new set of environmental circumstances, the phenotypic response to environmental stimuli, i.e. the reaction norm (Scheiner 1993), needs to change genetically (Nussey et al. 2005) Examples of changes in genotypes are much rarer than reports on phenotypic change. One such an example is the pitcher plant mosquito's (*Wyeomyia smithii*) response to photoperiod, which has changed over a five-year period (Bradshaw and Holzapfel 2001). Also, a red squirrel (*Tamiasciurus hudsonicus*) population in northern Canada has shown marked genetic changes within a ten year study period (Réale et al. 2003), but the change due to phenotypic plasticity was more pronounced. This is in sharp contrast with the response in other species, like a great tit population in the Netherlands, where the birds have hardly changed their laying date in a thirty-year period (Gienapp et al. 2006) and they cannot keep up with the rate of change of maximum food availability, this despite the occurrence of genetic variation in – and selection on - reaction norm of egg laying (Nussey et al. 2005).

Here, we investigate changes in the reaction norm of winter moth (*Operophtera brumata*) egg hatching in response to temperature. Winter moths have an annual life cycle,

and in spring the larvae depend on young foliage to feed on. Leaves from deciduous trees are only suitable as food during a short period of time; hence the timing of egg hatch relative to bud opening is crucial (Van Asch and Visser 2007). Even a few days' difference between egg hatch and oak (*Quercus robur*) bud opening has marked fitness consequences (Feeny 1970; Tikkanen and Julkunen-Tiitto 2003; Van Asch and Visser 2007). Both oak bud opening and winter moth egg hatching are temperature dependent, and the dates of both have advanced over the past 25 years (Visser and Holleman 2001). However, winter moth egg hatch has responded more strongly to the increase in temperature, leading to a decrease in synchronization between oak and winter moth in the Netherlands (but see Buse and Good 1996 for an English population). In this case the phenotypic response of the eggs to temperature is not sufficient to remain in synchrony with the host plant; adaptation is only possible if the reaction norm of egg hatching versus temperature changes. We expect eggs to become less responsive to temperature, as their current response is too strong (Van Asch et al. 2007).



ate

(April) versus temperature over the period 2000-2005. (A) Predictions (2000-2005) are based on the genetic (co)variation estimates in offspring of females caught in Oosterhout, Doorwerth and Warnsborn (autumn 2000)(Van Asch et al. 2007), in combination with the actual observed temperatures and oak bud opening within each forest (determining the selection pressure). Observed changes in reaction norms (B) are based on experimentally determined changes (2000 and 2005). Temperature (°C) is the mean temperature from January 1st until March 31st. Lines represent the changes in reaction norm over time (2000: open symbols, dashed lines; 2005: filled symbols, solid line). Forests are denoted by different colors (Doorwerth: black; Oosterhout: light gray; Warnsborn: dark gray). Arrows indicate changes over time.

Given current selection pressures and estimates of genetic (co)variation in the reaction norm (Van Asch et al. 2007), we can make clear predictions of the rate at which the reaction norm should change (Figure 1A). Our model predicts that the elevation of the reaction norm should have increased between 2000 and 2005 by 3-6 days, depending upon the temperature (Figure 1A). The largest change should have been at the higher temperatures (change in slope of the reaction norm). The genetic estimates we use were obtained from offspring of moths caught in 2000. Since then, we have been studying the

same populations, so we can compare the predictions of the model with the actual, observed changes in reaction norm.

We use two different approaches to test these predicted changes in reaction norm elevation (see Methods). We first use an experimental setup to determine the changes in reaction norm. We divided eggs of female winter moths over three temperature treatments and thus could determine the reaction norm of each female from the egg hatch dates of her offspring at different temperatures. We performed this experiment twice (2000 and 2005) in order to determine the changes in reaction norm over time. In 2005 eggs hatched five days later than in 2000 for all temperatures (Figure 1B, Table 1). The reaction norm only changed in elevation, not in slope. However, the predicted change within this time span is also small, and the observed slope of the reaction norm in 2005 did not differ from the predicted slope ($F_{1,73}$=3.35, ns).

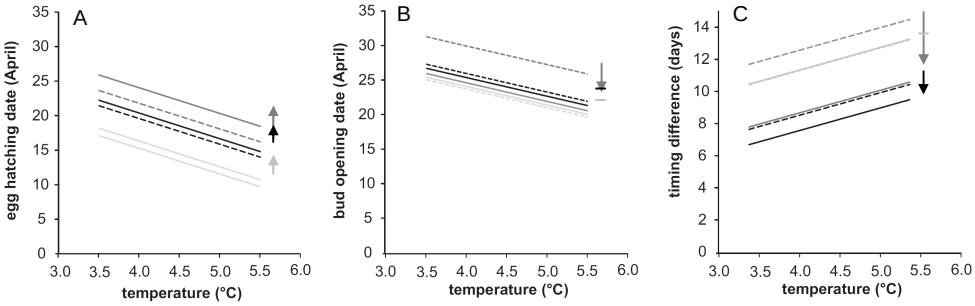


Figure 2 Observed changes over time in (A) egg hatching date (April), (B) oak bud opening date (April) and (C) degree of synchronization between bud opening and egg hatching (days) in three different Dutch forests. Temperature (°C) is the mean temperature from January 1st until March 31st. Lines represent the changes over time (2000 dashed lines; 2005 solid lines), estimated in a mixed model including temperature and year. Forests are denoted by different colors (Doorwerth black lines; Oosterhout light gray lines; Warnsborn dark grey lines). Arrows indicate changes over time.

In addition, we use a long-term data set (1995-2006) on winter moth egg hatching (common garden setup, eggs were kept together in a outdoor insectarium at the NIOO-KNAW in Heteren; eggs came from females caught in three forests in the Netherlands). As predicted, the elevation of the reaction norm has indeed increased (Figure 2A, Table 1), by 2.5 (Oosterhout forest), 1.9 (Doorwerth forest) and 5.2 (Warnsborn forest) days over the study period, which means an increase of 0.16 – 0.43 day per year (see Appendix 1 for among area differences).

In our long term data on winter moth populations we also monitor opening of oak buds within the forests. These data show that the observed change in reaction norm elevation has indeed led to a better synchrony between winter moth egg hatching and the bud opening of its host plant, the oak (Figure 2, Table S1) over the period 1995-2006, especially in

Warnsborn forest. Oak bud opening cannot have changed genetically, as we used the same focal trees throughout the study period.

We have shown here that the (elevation of) reaction norm can respond to changing selection pressures due to climate change within a relatively short period (5 years), resulting in a better synchrony between an insect herbivore and its host plant. Both the experimentally determined changes and the long term observations show an increase in hatching date over time, and, although the change was slightly stronger in the experimental setup, both are close to the predicted changes of about one day per year during the study period. Establishing this rate of genetic change is crucial, rather than determining whether or not a species can adapt, as the current climate is predicted to change much more rapidly than ever before (IPCC 2007). The pivotal question is whether species can keep up with the rate of climate change, and winter moths are an example where environmental change does lead to a response to selection within a few generations.

Material and Methods

Predicted changes in winter moth egg hatch

We based our predictions on the model described by (Van Asch et al. 2007). In this model we use estimations of genetic (co)variation and selection pressures in winter moth populations to predict evolutionary changes in the timing of egg hatching. The genetic variation was estimated using offspring of females caught in autumn 2000 in the same forests as those used in the present study.

Oak and winter moth phenology

We collected data on both oak bud opening date and winter moth egg hatch date during twelve years (1995-2006) in three forests in the area around Arnhem, the Netherlands (Oosterhout (05°50’ E, 51°55’ N), Doorwerth (05°48’ E, 51°59’ N) and Warnsborn (05°50’ E, 52°05’ N)).

Eggs were collected from female winter moths caught in the forest; these eggs were then kept in an outdoor insectarium in Heteren (05°44’ E, 51°57’ N) until egg hatch. Each autumn we used insect traps to catch female moths on oak trees. In each forest we put up traps at several sites (Doorwerth and Warnsborn three sites, Oosterhout six sites), and on each site we used four trees (each year the same ones). Females were kept individually in plastic containers and were provided with a roll of tissue paper on which to lay their eggs. Each year, we randomly selected 15-40 broods from each forest (except Warnborn 2003, when only three females were caught); the eggs were then stored in the outdoor insectarium until egg hatch the following spring. Hatching was checked three times a week, and from this we determined the median egg hatching date per brood. Eggs thus kept in the outdoor insectarium hatched at the same date as eggs kept within the forests (Visser and Holleman 2001; M. van Asch, unpublished data).

Oak bud opening was determined for ten trees per site per forest, including the trees where we caught the female moths. We checked oak bud opening two to three times a week, and bud opening date was determined as the date when half of the buds in the crown of the tree had opened.

We calculated the degree of synchronization as the (average) bud opening date minus the (average) egg hatching date, in each year for each forest separately.

Analyses were done using a mixed model procedure in SAS (v8), with forest of origin as a factorial explanatory variable, and temperature (mean from January 1st until March 31st) and year as continuous variables. To correct for the fact that we have multiple (non-independent) observations within a year, we also included year as a random factor.

Winter moth reaction norm

We also repeatedly determined egg hatching in response to different temperature treatments (reaction norm) in different years, to check experimentally whether the reaction norm changed over time. Eggs of female winter moths were divided over three temperature treatments (at least 25 eggs per treatment per female, 14-30 females). Eggs were checked three times a week for hatching, and median egg hatching date was determined for each female at each treatment.

Rather than using constant artificial temperatures in each treatment, we used fluctuating temperatures mimicking specific years (1973, 1983 and 1999, average temperatures from January 1st until April 1st of 3.5°C, 4.5°C and 5.5°C, respectively) (Visser and Holleman 2001). These provided a more natural temperature pattern than using constant temperatures. The temperature pattern in these years was mimicked in climate cabinets (SANYO Incubator MIR-553) using three phases a day: 6 hours at the daily minimum temperature, 12 hours at the mean of the daily maximum and daily average temperature and 6 hours at the daily average temperature. These temperatures were changed three times a week according to the temperatures in the mimicked year (Visser and Holleman 2001).

We repeated the experiment in two years (2000 and 2005) and with eggs from different forests (Oosterhout, Doorwerth and Warnsborn).

Several factors affecting egg hatch can differ in the two years. To control for (genetic) within-year differences, we used eggs from females caught around the peak date in both years. This prevented the use of different subsets of the population in the different years (i.e., early emerging females in one year and late emerging ones in the other year). In 2000 mean catching date was 29 (±0.9 s.e.m.) November. However, in 2005 mean catching date in Oosterhout was marginally later (2 (±1.3) December) than in 2000. To control for laying date effects, we ensured that a subset of the Oosterhout females in 2005 were caught on exactly the same date and within the same sites as in 2000. Analysis with only this subset of females gave exactly the same results as analysis of the full dataset. Therefore, only the results of the full dataset are shown here. Neither laying date nor composition of the females could thus explain the difference in hatching date between years.

Analyses were done using a mixed model procedure in SAS (v8). Year and forest of origin were the factorial explanatory variables, while the mean temperature from January 1st until March 31st in the temperature treatments as a continuous explanatory variable. Brood was included in the model as a random factor.

Appendix 1

Differences among forests

When comparing the different forests in the long term dataset (1995-2006), Warnsborn eggs changed more than Oosterhout and Doorwerth eggs (interaction forest*year, Table S1). We can understand this when comparing the composition of trees within the forests. Although all three forests consist predominantly of mature oak trees, in both Oosterhout and Doorwerth other trees and shrubs such as maple (*Acer pseudoplantanus*) and red oak (*Quercus rubra*) are present underneath the oak trees. These may serve as an alternative food source for the larvae, thereby reducing selection (and thus genetic changes) within those forests. In Warnsborn, there are hardly any alternative food plants, and thus the selection pressure is presumably stronger there.

In the experimental setup, Oosterhout eggs hatched earlier than both Doorwerth and Warnsborn eggs ($F_{1,97.1}$=31.77, p<0.0001 and $F_{1,76.1}$=14.7, p=0.0003, respectively), which did not differ in hatching date ($F_{1,78.1}$=1.17, p=0.28). In contrast with the long term observational data, the increase in hatching date in the experiment was the same in all three forests (interaction forest*year: $F_{2,124}$=0.16, p=0.85).

The observed changes are all in the elevation of the reaction norm, not in the slope, i.e. the moths did not become more (or less) phenotypically plastic. However, the predicted change in slope is actually smaller than the amount of change that we could actually have detected with our long-term data set. In the experimental setup the slope also remained the same. However, this slope was not significantly different from the predicted slope.

Oak bud opening remained the same over time in two of the three forests (Oosterhout and Doorwerth), but showed a slight advancement over time in Warnsborn (Table S1). This cannot be due to genetic differences, because we used the same focal trees during the whole study period. Unlike the eggs, which were kept in a common garden setup at the NIOO-KNAW in Heteren, the trees were still located within each forest, with potentially slightly different environments. Small scale temperature variations between the three areas could be one possible explanation for these differences, because we used one temperature measurement for all three areas. However, temperature did not differ consistently between the forests during two years of temperature measurements. Eggs hatched at the same time in Heteren as in their forest of origin (Visser and Holleman 2001; M van Asch, unpublished data).

Appendix 2

Parameter estimates in the models (table 1).

Table 1: Remaining parameters in the models explaining egg hatching in the experiment, and egg hatching, oak bud opening and degree of synchronization between hatching and bud opening in the years 1995-2006 in three different forests (Oosterhout (OH), Doorwerth (DO) and Warnsborn (WA)). Yearly estimates represent the changes between the experimental years (2000 and 2005) in the experimental setup, in the observational data forest*year estimates represent differences per year per forest (slope of the reaction norm).

Data	Effect	Num	Den	F	p	Estimate (± s.e.m.)
Egg hatching	Year	1	126	50.2	<0.0001	4.9 (± 0.7)
(experiment)	Temperature	1	257	8203	<0.0001	-11.3 (± 0.1)
	Forest	2	126	16.5	<0.0001	OH: 75.7 (± 0.9)
						DO: 80.0 (± 0.9)
						WA: 79.0 (± 1.0)
Egg hatching	Year	1	9.83	1.71	0.22	
(1995-2006)	Temperature	1	10.2	30.3	0.0002	-3.7 (± 0.7)
	Forest	2	14.3	4.45	0.031	OH: 29.1 (± 1.2)
						DO: 33.6 (± 1.2)
						WA: 34.4 (± 4.1)
	Forest*year	2	14.3	4.41	0.032	OH: 0.21 (± 0.1)
						DO: 0.16 (± 0.1)
						WA 0.43 (± 0.3)
Oak bud opening	Year	1	8.62	0.48	0.50	
(1996-2006)	Temperature	1	9.44	4.97	0.051	-2.5 (± 1.1)
	Forest	2	16.4	8.41	0.003	OH: 3.5 (± 2.8)
						DO: 36.6 (± 2.8)
						WA: 44.6 (± 6.5)
	Forest*year	2	16.3	4.15	0.035	OH: 0.07 (± 0.4)
						DO: -0.11 (±0.4)
						WA: -0.99 (± 0.5)
Synchronization	Year	1	8.78	0.80	0.39	
(1996-2006)	Temperature	1	9.64	3.95	0.07	1.4 (± 0.8)
	Forest	2	16.5	5.24	0.017	OH: 5.4 (± 2.2)
						DO: 3.6 (± 2.2)
						WA: 10.6 (± 4.7)
	Forest*year	2	16.4	3.36	0.059	OH: 0.01 (± 0.3)
						DO:-0.19 (± 0.3)
						WA: -0.78 (± 0.4)

Chapter 7: Summarizing discussion

Adapting to a changing environment

One of the main reasons for starting this project, was the observation that synchrony between oak bud opening and winter moths egg hatch in the Netherlands seemed to have decreased in the past 30 years (Visser and Holleman 2001), and especially in warm springs eggs tend to hatch before the oak buds open.

Maintaining synchrony wit the underlying trophic level is a potential problem for many species under a changing climate, as there is no a priori reason why different species within a food chain would shift their phenology at the same rate (Visser and Both 2005). Relatively small effects of climate change can have profound consequences for the ecosystem if species that depend on one another, such as a herbivore and its host plant, react differently to an increase in temperature, thus leading to asynchrony or a mismatch (Visser and Holleman, 2001; Bale et al., 2002; Stenseth and Mysterud, 2002; Visser et al., 2004).

Aim of my PhD project was to identify and understand the different factors affecting synchrony between an insect herbivore and its host plant, using winter moth (*Operophtera brumata*) on oak (*Quercus robur*) as a study system. The winter moth – oak system is an example where climate change can potentially lead to problems, and is thus a good model system to use. There is only a very short period in which the herbivore can forage on its host, and because the selection on synchrony will be strong and because generation time of the herbivore is much shorter that that of its host, this creates the potential for rapid adaptation. Other (practical) advantage of this study system are that is an already relatively well studied system, both oak and winter moth are common in large areas of Europe, and winter moths are relatively well suited to perform experiments with. To understand why synchrony may be affected, I used a physiological approach, looking at the temperature response of winter moth eggs. I have also looked at genetic and environmental (maternal) effects on this temperature response, and fitness consequences of (a)synchrony. Combining all these things, I was then able to predict the consequences of climate change, and to look at the different ways a species may have to deal with these effects.

Not only the fact that the climate is changing, but also the rate of change is a cause for concern: the current climate is predicted to change much more rapidly than ever before (IPCC 2007). Establishing the rate of (genetic) change is then crucial, because the pivotal question is whether species can keep up with the rate of climate change. In the last two chapters I therefore predict rates of change in a changing environment, and I observe whether there already have been any changes in phenology.

Environmental cues determining phenology

Synchrony between an insect and its host plant is determined by the phenology of both insect and host. Therefore, to understand the proximate factors affecting synchrony, we needed to understand the mechanisms determining both insect and host plant phenology.

Oak trees in the Netherlands first go through a rest phase, and then enter an active phase (Kramer 1994). The transition from rest phase to active phase is temperature dependent, and is reached sooner at low (optimum -0.8 °C) temperature than at high temperature. In the Netherlands, this transition is usually achieved in late March or early April. Development during the active phase is, in contrast, faster at high temperature.

Degree-day models are commonly used to describe insect phenology, because they often correlate well with observed data. However, predictions are only reliable for the populations and temperature range and patterns that were used to estimate the model parameters. Under environmental change, not only the average temperature may increase, but the whole temperature pattern could change, and temperatures may not increase uniformly over the year. Under those new conditions the predictions of a purely correlational model may no longer be valid. Also temperatures may move outside the temperature range under which development rate is a linear function of temperature (an assumption of degree-day models). It is therefore crucial to have a more mechanistic model. Moreover, a good understanding of the physiological processes is important to understand why synchrony may become disrupted, as for instance host plant and insect may be sensitive to temperature during different periods in the year. At the temperature extremes egg development rate was indeed no longer a linear function of temperature, but there is a relatively large temperature range where development rate is a more or less linear function of temperature. A nonlinear, physiologically based model fitted my experimental data better than a simple, linear, degree-day model (chapter 3). However, under more natural conditions neither model fitted the data well. Both the linear and the nonlinear model assume a constant development rate. Although still in need of further experimental study, incorporation of a development dependency of development improved the fit of the model considerably. An alternative degree-day model (Kimberling and Miller 1988; Visser and Holleman 2001) included the number of frost days. Although tested experimentally in a Canadian population (Kimberling and Miller 1988), this model is solely used as a correlative model in the Dutch population (Visser and Holleman 2001), and it does not seem to reflect a causal mechanism, because experimentally increasing the length or the amount of cold merely delays egg hatch, instead of advancing it.

When comparing egg and oak bud development, some differences become apparent. Although egg development rate increases over time, even early on an increase in temperature results in an increase in development rate. At that time oak buds have not yet started their development, but they are still in the rest phase. Under higher winter temperatures it takes a longer time before the transition can be made to the active phase (where development rate is then temperature dependent). This, then, can explain why an increase in temperature leads to a disruption between oak and winter moth phenology: warm spring temperatures advance both oak and moth phenology, but warm winter temperatures only advance the moth’s phenology, leading too an advancement in egg hatching that is too large in warm years

Maternal effects on offspring emergence

Maternal effects may play an important role in shaping the life history of organisms. They can affect offspring size and number (e.g., Rossiter 1996; Mousseau and Fox 1998), and determine the onset and termination of lifecycle stages (Roach and Wulff 1987; Mousseau and Dingle 1991; Etterson and Galloway 2002; Gorman and Williams 2005). Although winter moth males can fly, females are wingless and do not disperse far. Thus, feeding conditions of the mother are a better predictor of feeding conditions for her offspring than are those of the father, one of the prerequisites for adaptive maternal effects to occur (Donohue and Smith 1998; Galloway 2005).

Maternal feeding conditions do affect herbivore development time. As the season progresses, oak leaves increase in tannin content and decrease in water and nitrogen content. The moment when the leaves become inedible may serve as a 'synchronisation' point: independent of how long larvae have been feeding, they should pupate at that time, as they cannot feed on the older leaves. If the pupal and egg period are then a fixed length, this may then be another factor affecting synchrony with the host.

Some across-generation effects of tannins do exist (Rossiter 1991), and leaf age does affect larval development time (Tikkanen and Lyytikainen-Saarenmaa 2002). In chapter 4 I show that maternal effects can shape herbivore phenology in an adaptive manner, affecting not only parental development time, but also egg hatching date of the offspring. Feeding on relatively old leaves (i.e., larva that hatched late relative to bud opening) reduced time until egg hatch in the offspring.

Maternal effects can either decrease or increase the rate of response to selection, and thus accelerate or slow down evolutionary change (Kirkpatrick and Lande 1989). If the maternal effect leads to a (non-genetic) adaptation, we expect selection pressure to decrease, and thereby also the rate of (genetic) adaptation. However, in this particular example this does not hold true because winter moths tend to hatch before oak bud opening. The resulting mortality is so high that hardly any larvae survive. Thus the relative importance of maternal feeding conditions in the present circumstances is – in this particular case- probably negligible.

Genetic determination of phenology

In general, it is assumed that in those cases were synchrony in emergence with the underlying trophic level is important, synchrony is maintained through strong selection on emergence date. In the case of the winter moths, strong selection acts on timing of egg hatching in relation to oak bud opening. Pupation weight, and hence fecundity, declines rapidly when feeding on older leaves, due to an increase in leaf toughness and defensive compounds and a decrease in water and nitrogen content. On the other hand, hatching before the oak buds open also has severe fitness consequences, as the larvae starve within a few days in the absence of food. Our results support previously published findings (Feeny 1970; Wint 1983; Tikkanen and Julkunen-Tiitto 2003) that only a few days' difference

between oak bud opening and egg hatching already greatly reduces fitness, in our case five days' difference reduced fitness by 30 – 90 % (hatching after and before bud opening, respectively, chapters 4 and 5). For selection to be effective in maintaining synchrony, the relevant trait has to be genetically determined, and sufficient variation in the trait should exist (Falconer and Mackay 1996). In the winter moths phenotypic adaptation has been reported, with moths being adapted to the phenology of individual oak trees (Van Dongen et al. 1997), that is, eggs from early developing oak trees also hatch relatively early. However, large temporal variation in environmental conditions exists between years. Thus, the environmental conditions may differ greatly between the different generations. Each year, under the environmental conditions in that year, synchrony between herbivore and host should be optimal. A way of looking at the genetic variation in phenotypic plasticity is to use a reaction norm approach. A reaction norm looks at the expressed phenotypes a certain genotype will have, depending upon the environmental conditions (Woltereck 1909; Scheiner 1993). In chapter 5 we estimate the genetic (co)variance in egg hatching date within and among temperature treatments. Heritability estimates were high, and genetic variation existed also in slope of the reaction norm, i.e. some individuals are more phenotypically plastic than others (Postma and van Noordwijk 2005; Nussey et al. 2007).
In the case of the winter moths there is both strong selection on and genetic variation in egg hatching reaction norm; we therefore expect to see an adaptive response in egg hatching. In chapter 5 we predict a rapid change in egg hatching phenology, resulting in a better – average- synchrony with oak bud development. Despite the existence of genetic variation in slope of the reaction norm, the predicted change is mainly in the elevation of the reaction norm. In addition to this, we did predict to see a slight change in slope of the reaction norm, resulting in slightly less phenotypically plastic eggs.

In chapter 6 we compare these predicted changes with the observed changes. We determined experimentally that reaction norm elevation has increased five days in the past five years (2000, 2005). This result was further supported by analysis of our long-term data (1995-2006), where we also found an increase in elevation of the reaction norm.

Relatively few studies have reported on climate change induced genetic changes. A mosquito has changed its response to photoperiod (Bradshaw and Holzapfel 2001), and a squirrel has advanced its breeding (Réale et al. 2003), and part of this change was due to genetic adaptation. As climate scenarios all predict that the climate will continue to change at an unprecedented rate (IPCC 2007), the rate at which species are able to adapt is of major concern. Both the squirrel and the mosquito study found evidence for genetic changes within a relatively short period (5-10 years). Our long term observations and our experimental study have a similar time span. Within that period we also observe clear changes as well. Moreover, the observed changes match the predicted amount of change.

One thing that did not fit with our predictions is that the reaction norm only changed elevation, the slope remained the same, despite genetic variation in reaction norm slope. The chance of observing such a change in slope in the long-term observations is slim, as the change in elevation was larger than that in slope. However, we also did not find evidence

for a change in the slope in the experimental data, where we should have been able to observe the change if it had occurred. The explanation is then that we did not find a change simply because it did not occur. Something similar is observed in *Bicyclus anynana* butterflies (Wijngaarden et al. 2002) that under artificial selection only changed in reaction norm elevation, despite genetic variation in and selection on slope. Correlations among traits may also prevent changes, as shown by the example of prairie plants (Etterson and Shaw 2001) that show slower than expected adaptation.

Another option available to generalist species is to switch host plants. Winter moths are a generalist species, they do also feed on other food plants (Watt and McFarlane 1991; Kerslake et al. 1998; Tikkanen and Lyytikainen-Saarenmaa 2002; Van Asch, unpublished observation), and thus they can in theory just move to another host plant with a more suitable phenology than oak trees. Comparison of different forests within the Netherlands suggests that that may indeed be the case. Our long term observations suggested that eggs in one of the forests (Warnsborn) have changed more than those in the other forests. An explanation could be the composition of the forests: although all three forests consist predominantly of mature oak trees, in both Oosterhout and Doorwerth there is a substantial part of the forest were also other trees and shrubs such as red oak (*Quercus rubra*) and maple (*Acer pseudoplatanus*) are present underneath the oak trees. These may serve as an alternative food source for the larvae, thereby reducing the selection (and thus the genetic changes) within those forests. In Warnsborn, there are far less alternative food plants, and thus the selection pressures are presumably stronger there. This would support the idea that –if possible- a generalist species like winter moths just switches towards another host plant. Although this seems a likely explanation, with our data we cannot test this, as we only have long term information on three forests. During one year I did collect information in several more forests (n=6, in total, unpublished data) with varying amounts of other plant species available to the larvae. On sites with many red oak (*Quercus rubra*) trees (20-50 %), winter moth eggs tended to hatch earlier as well (red oaks developed about a week before the European oaks did). This supports the idea that the moths can become adapted to the phenology of their host plant (Tikkanen and Lyytikainen-Saarenmaa 2002). In a forest with a more mixed composition the moths may become adapted to a combination of host plants, and thus decrease the synchrony with oak.

The kind of model we used may be particularly useful in predicting the effects of environmental changes, to gain insight in the expected amount and rate of change, because its predictions do seem to match the observed changes. When the amount of genetic variation and the selection pressures are known, this is a relatively simple model to make these predictions. However, a certain amount of caution is necessary, since even if species have a clear potential to adapt to climate change, as in the case of *O. brumata*, there may still be other factors hampering a rapid response to selection. Most particularly, in our model populations could not go extinct. While this may be a reasonable assumption when modelling a species like winter moth, with a short generation time and a high reproduction potential, this problem is much larger for longer lived species with fewer offspring.

Moreover, the potential to shift towards another host plant is clearly something that is only an option open to generalist species, and the specialist species do not have such an escape if their timing no longer matches with their host plant. Indeed in general specialist, non-dispersing species are expected to have much more problems (e.g., Thomas et al. 2004) in adapting to a changing climate than generalist species or species with a high mobility.

Conclusion

Synchrony between different trophic levels is affected by many different processes. In this thesis I have shown that both environmental and genetic effects play a major role in shaping herbivore emergence. In fluctuating environments it is imperative to understand and integrate physiological processes determining insect and host emergence. Only then is it possible to study the genetic variation in these mechanisms. Not only in rapidly changing environments, but also from a more general evolutionary perspective a major goal is to predict genetic changes under directional selection. In this thesis I show that combining estimates of genetic variation in reaction norms with selection pressures gives a good prediction of a species' response to selection.

References

Akimoto, S. I. (1998). "Heterogeneous selective pressures on egg-hatching time and the maintenance of its genetic variance in a Tetraneura gall-forming aphid." *Ecological Entomology* **23**(3): 229-237.

Andresen, J. A., D. G. McCullough, B. E. Potter, C. N. Koller, L. S. Bauer, D. P. Lusch and C. W. Ramm (2001). "Effects of winter temperatures on gypsy moth egg masses in the Great Lakes region of the United States." *Agricultural and Forest Meteorology* **110**(2): 85-100.

Bale, J. S., G. J. Masters, I. D. Hodkinson, C. Awmack, T. M. Bezemer, V. K. Brown, J. Butterfield, A. Buse, J. C. Coulson, J. Farrar, J. E. G. Good, R. Harrington, S. Hartley, T. H. Jones, R. L. Lindroth, M. C. Press, I. Symrnioudis, A. D. Watt and J. B. Whittaker (2002). "Herbivory in global climate change research: direct effects of rising temperature on insect herbivores." *Global Change Biology* **8**(1): 1-16.

Barker, J. F. and J. W. Enz (1993). "Development of laboratory-reared banded sunflower moth, Cochylis-Hospes Walsingham (Lepidoptera, Cochylidae), in relation to temperature." *Journal of the Kansas Entomological Society* **66**(4): 420-426.

Bergant, K., S. Trdan, D. Znidarcic, Z. Crepinsek and L. Kajfez-Bogataj (2005). "Impact of climate change on developmental dynamics of Thrips tabaci (Thysanoptera : Thripidae): Can it be quantified?" *Environmental Entomology* **34**(4): 755-766.

Bonnemaison, L. (1971). "Particularites de la croissance embryonnaire et de la daipause nymphale chez la Cheimatobie (*Operophtera brumata* L.)." *Bulletin de la Societe entomologique de France* **76**: 123-130.

Both, C., S. Bouwhuis, C. M. Lessells and M. E. Visser (2006). "Climate change and population declines in a long-distance migratory bird." *Nature* **441**(7089): 81-83.

Bradshaw, W. E. and C. M. Holzapfel (2001). "Genetic shift in photoperiodic response correlated with global warming." *Proceedings of the National Academy of Sciences of the United States of America* **98**(25): 14509-14511.

Bradshaw, W. E. and C. M. Holzapfel (2006). "Climate change - Evolutionary response to rapid climate change." *Science* **312**(5779): 1477-1478.

Bryant, S. R., J. S. Bale and C. D. Thomas (1999). "Comparison of development and growth of nettle-feeding larvae of Nymphalidae (Lepidoptera) under constant and alternating temperature regimes." *European Journal of Entomology* **96**(2): 143-148.

Bryant, S. R., C. D. Thomas and J. S. Bale (2002). "The influence of thermal ecology on the distribution of three nymphalid butterflies." *Journal of Applied Ecology* **39**(1): 43-55.

Buse, A., S. J. Dury, R. J. W. Woodburn, C. M. Perrins and J. E. G. Good (1999). "Effects of elevated temperature on multi-species interactions: the case of Pedunculate Oak, Winter Moth and Tits." *Functional Ecology* **13**: 74-82.

Buse, A. and J. E. G. Good (1996). "Synchronization of larval emergence in winter moth (*Operophtera brumata* L) and budburst in pedunculate oak (*Quercus robur* L) under simulated climate change." *Ecological Entomology* **21**(4): 335-343.

Buse, A., J. E. G. Good, S. Dury and C. M. Perrins (1998). "Effects of elevated temperature and carbon dioxide on the nutritional quality of leaves of oak (*Quercus robur* L.) as food for the Winter Moth (*Operophtera brumata* L.)." *Functional Ecology* **12**(5): 742-749.

Cannell, M. G. R. and R. I. Smith (1983). "Thermal Time, Chill Days and Prediction of Budburst in Picea sitchensis." *Journal of Applied Ecology* **20**(3): 951-963.

Cannell, M. G. R. and R. I. Smith (1986). "Climatic warming, spring budburst and forest damage on trees." *Journal of Applied Ecology* **23**(1): 177-191.

Carroll, A. L. and D. T. Quiring (1994). "Intratree Variation in Foliage Development Influences the Foraging Strategy of a Caterpillar." *Ecology* **75**(7): 1978-1990.

Connett, J. F., L. M. Wilson, J. P. McCaffrey and B. L. Harmon (2001). "Phenological synchrony of *Eustenopus villosus* (Coleoptera : Curculionidae) with *Centaurea solstitialis* in Idaho." *Environmental Entomology* **30**(2): 439-442.

Crawley, M. J. and M. Akhteruzzaman (1988). "Individual variation in the phenology of oak trees and its consequences for herbivorous insects." *Functional Ecology* **2**(3): 409-415.

Denlinger, D. L. (2002). "Regulation of diapause." *Annual Review of Entomology* **47**: 93-122.

Dewar, R. C. and A. D. Watt (1992). "Predicted changes in the synchrony of larval emergence and budburst under climatic warming." *Oecologia* **89**(4): 557-559.

Diss, A. L., J. G. Kunkel, M. E. Montgomery and D. E. Leonard (1996). "Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L." *Oecologia* **106**(4): 470-477.

Donohue, K. (1999). "Seed dispersal as a maternally influenced character: Mechanistic basis of maternal effects and selection on maternal characters in an annual plant." *American Naturalist* **154**(6): 674-689.

Donohue, K. and J. Smith (1998). Maternal environmental effects in plants: adaptive plasticity? *Maternal effects as adaptations*. T. A. Mousseau and C. W. Fox. Oxford, University Press**:** 137-158.

Du Merle, P. (1999). "Egg development and diapause: ecophysiological and genetic basis of phenological polymorphism and adaptation to varied hosts in the green oak tortrix, *Tortrix viridana* L-(Lepidoptera : Tortricidae)." *Journal of Insect Physiology* **45**(6): 599-611.

Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Changnon, T. R. Karl and L. O. Mearns (2000). "Climate extremes: Observations, modeling, and impacts." *Science* **289**(5487): 2068-2074.

Embree, D. G. (1970). "The diurnal and seasonal pattern of hatching of winter moth eggs, *Operophtera brumata* (Geometridae: Lepidoptera)." *Canadian Entomologist* **102**: 759-768.

Esch, M. (2005). "ECHAM4_OPYC_SRES_B2: 110 years coupled B2 run 6H values. Doi: 10.1594/WDCC/EH4_OPYC_SRES_B2." Retrieved 2005-02-09, from http://cera-www.dkrz.de/WDCC/doi/Experiment.jsp?acronym=EH4_OPYC_SRES_B2.

Etterson, J. R. and L. E. Galloway (2002). "The influence of light on paternal plants in *Campanula americana* (Campanulaceae): Pollen characteristics and offspring traits." *American Journal of Botany* **89**(12): 1899-1906.

Etterson, J. R. and R. G. Shaw (2001). "Constraint to adaptive evolution in response to global warming." *Science* **294**(5540): 151-154.

Falconer, D. S. and T. F. C. Mackay (1996). *Introduction to Quantitative Genetics*. Harlow, Addison Wesley Longman Ltd.

Feeny, P. (1968). "Effect of oak leaf tannins on larval growth of the winter moth *Operophtera brumata*." *Journal of Insect Physiology* **14**(6): 805-817.

Feeny, P. (1970). "Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars." *Ecology* **51**(4): 565-58.

Feeny, P. (1976). "Plant apparency and chemical defense." *Recent Advances in Phytochemisty* **10**: 1-40.

Galloway, L. F. (2005). "Maternal effects provide phenotypic adaptation to local environmental conditions." *New Phytologist* **166**(1): 93-99.

Gienapp, P., E. Postma and M. E. Visser (2006). "Why breeding time has not responded to selection for earlier breeding in a songbird population." *Evolution* **60**(11): 2381-2388.

Gorman, K. B. and T. D. Williams (2005). "Correlated evolution of maternally derived yolk testosterone and early developmental traits in passerine birds." *Biology Letters* **1**(4): 461-464.

Got, B., J. M. Labatte and S. Piry (1996). "European corn borer (Lepidoptera: Pyralidae) development time model." *Environmental Entomology* **25**(2): 310-320.

Grafton-Cardwell, E. E., P. Gu and G. H. Montez (2005). "Effects of temperature on development of vedalia beetle, *Rodolia cardinalis* (Mulsant)." *Biological Control* **32**(3): 473-478.

Gray, D. R., F. W. Ravlin and J. A. Braine (2001). "Diapause in the gypsy moth: a model of inhibition and development." *Journal of Insect Physiology* **47**(2): 173-184.

Gray, D. R., F. W. Ravlin, J. Regniere and J. A. Logan (1995). "Further Advances toward a Model of Gypsy-Moth (Lymantria-Dispar (L)) Egg Phenology - Respiration Rates and Thermal Responsiveness During Diapause, and Age-Dependent Developmental Rates in Postdiapause." *Journal of Insect Physiology* **41**(3): 247-256.

Hakkinen, R., T. Linkosalo and P. Hari (1998). "Effects of dormancy and environmental factors on timing of bud burst in Betula pendula." *Tree Physiology* **18**(10): 707-712.

Hanninen, H. (1991). "Does Climatic Warming Increase the Risk of Frost Damage in Northern Trees." *Plant Cell and Environment* **14**(5): 449-454.

Harrison, S. (1994). "Resources and Dispersal as Factors Limiting a Population of the Tussock Moth (*Orgyia-Vetusta*), a Flightless Defoliator." *Oecologia* **99**(1-2): 27-34.

Haukioja, E. (2005). "Plant defenses and population fluctuations of forest defoliators: mechanism-based scenarios." *Annales Zoologici Fennici* **42**(4): 313-325.

Haukioja, E. and S. Neuvonen (1988). Insect population dynamics and induction of plant resistance: The testing of hypotheses. *Insect outbreaks*. P. Barbosa and J. C. Schultz. London, Academic Press, Inc.**:** 411-432.

Haukioja, E., V. Ossipov and K. Lempa (2002). "Interactive effects of leaf maturation and phenolics on consumption and growth of a geometrid moth." *Entomologia Experimentalis Et Applicata* **104**(1): 125-136.

Hill, J. K. and I. D. Hodkinson (1995). "Effects of Temperature on Phenological Synchrony and Altitudinal Distribution of Jumping Plant Lice (Hemiptera, Psylloidea) on Dwarf Willow (*Salix Lapponum*) in Norway." *Ecological Entomology* **20**(3): 237-244.

Holliday, N. J. (1985). "Maintenance of the Phenology of the Winter Moth (Lepidoptera, Geometridae)." *Biological Journal of the Linnean Society* **25**(3): 221-234.

Huey, R. B. and J. G. Kingsolver (1989). "Evolution of Thermal Sensitivity of Ectotherm Performance." *Trends in Ecology & Evolution* **4**(5): 131-135.

Hunter, A. F. (1993). "Gypsy moth population sizes and the window of opportunity in spring." *Oikos* **68**(3): 531-538.

Hunter, M. D. (1990). "Differential susceptibility to variable plant phenology and its role in competition between 2 insect herbivores on oak." *Ecological Entomology* **15**(4): 401-408.

Hunter, M. D., A. D. Watt and M. Docherty (1991). "Outbreaks of the Winter Moth on Sitka Spruce in Scotland Are Not Influenced by Nutrient Deficiencies of Trees, Tree Budburst, or Pupal Predation." *Oecologia* **86**(1): 62-69.

Hurrell, J. W., Y. Kushnir and M. Visbeck (2001). "Climate - The North Atlantic oscillation." *Science* **291**(5504): 603-605.

IPCC (2001). Synthesis Report. *IPCC Third Assessment Report: Climate Change 2001:*. R. T. Watson. Geneva**:** 1-34.

IPCC (2007). Summary for Policymakers. *WGI Fourth Assessment Report 2007*. Geneva**:** 1-18.

Ivashov, A. V., G. E. Boyko and A. P. Simchuk (2002). "The role of host plant phenology in the development of the oak leafroller moth, Tortrix viridana L. (Lepidoptera : Tortricidae)." *Forest Ecology and Management* **157**(1-3): 7-14.

Judd, G. J. R., G. H. Whitfield and H. E. L. Maw (1991). "Temperature-Dependent Development and Phenology of Pepper Maggots (Diptera, Tephritidae) Associated with Pepper and Horsenettle." *Environmental Entomology* **20**(1): 22-29.

Julkunen-Tiitto, R., H. Haggman, P. J. Aphalo, A. Lavola, R. Tegelberg and T. Veteli (2005). "Growth and defense in deciduous trees and shrubs under UV-B." *Environmental Pollution* **137**(3): 404-414.

Julkunen-Tiitto, R. and S. Sorsa (2001). "Testing the drying methods for willow flavonoids, tannins and salicylates." *Journal of Chemical Ecology* **27**(4): 779-789.

Kaitaniemi, P., K. Ruohomaki and E. Haukioja (1997). "Consequences of defoliation on phenological interaction between *Epirrita autumnata* and its host plant, Mountain Birch." *Functional Ecology* **11**(2): 199-208.

Kawecki, T. J. and D. Ebert (2004). "Conceptual issues in local adaptation." *Ecology Letters* **7**(12): 1225-1241.

Kerslake, J. E. and S. E. Hartley (1997). "Phenology of winter moth feeding on common heather: Effects of source population and experimental manipulation of hatch dates." *Journal of Animal Ecology* **66**(3): 375-385.

Kerslake, J. E., S. J. Woodin and S. E. Hartley (1998). "Effects of carbon dioxide and nitrogen enrichment on a plant- insect interaction: the quality of *Calluna vulgaris* as a host for *Operophtera brumata*." *New Phytologist* **140**(1): 43-53.

Kim, D. S., J. H. Lee and M. S. Yiem (2000). "Spring emergence pattern of Carposina sasakii (Lepidoptera : Carposinidae) in apple orchards in Korea and its forecasting models based on degree-days." *Environmental Entomology* **29**(6): 1188-1198.

Kim, D. S., J. H. Lee and M. S. Yiem (2001). "Temperature-dependent development of *Carposina sasakii* (Lepidoptera : Carposinidae) and its stage emergence models." *Environmental Entomology* **30**(2): 298-305.

Kimberling, D. N. and J. C. Miller (1988). "Effects of Temperature on Larval Eclosion of the Winter Moth, *Operophtera-Brumata*." *Entomologia Experimentalis Et Applicata* **47**(3): 249-254.

Kirkpatrick, M. and R. Lande (1989). "The evolution of maternal characters." *Evolution* **43**(3): 485-503.

Klemola, T., S. Hanhimaki, K. Ruohomaki, J. Senn, M. Tanhuanpaa, P. Kaitaniemi, H. Ranta and E. Haukioja (2003). "Performance of the cyclic autumnal moth, *Epirrita autumnata*, in relation to birch mast seeding." *Oecologia* **135**(3): 354-361.

Klemola, T., K. Ruohomaki, M. Tanhuanpaa and P. Kaitaniemi (2003). "Performance of a spring-feeding moth in relation to time of oviposition and bud-burst phenology of different host species." *Ecological Entomology* **28**(3): 319-327.

Komatsu, T. and S. Akimoto (1995). "Genetic Differentiation as a Result of Adaptation to the Phenologies of Individual Host Trees in the Galling Aphid *Kaltenbachiella-Japonica*." *Ecological Entomology* **20**(1): 33-42.

Kramer, K. (1994). "A modeling analysis of the effects of climatic warming on the probability of spring frost damage to tree species in the Netherlands and Germany." *Plant Cell and Environment* **17**(4): 367-377.

Kramer, K. (1994). "Selecting a model to predict the onset of growth of Fagus Sylvatica." *Journal of Applied Ecology* **31**(1): 172-181.

Kramer, K. (1995). "Phenotypic plasticity of the phenology of 7 European tree species in relation to climatic warming." *Plant Cell and Environment* **18**(2): 93-104.

Kumral, N. A., B. Kovanci and B. Akbudak (2005). "Pheromone trap catches of the olive moth, *Prays oleae* (Bern.) (Lep., Plutellidae) in relation to olive phenology and degree-day models." *Journal of Applied Entomology* **129**(7): 375-381.

Kuokkanen, K., P. Niemela, J. Matala, R. Julkunen-Tiitto, J. Heinonen, M. Rousi, H. Henttonen, J. Tahvanainen and S. Kellomaki (2004). "The effects of elevated CO2 and temperature on the resistance of winter-dormant birch seedlings (*Betula pendula*) to hares and voles." *Global Change Biology* **10**(9): 1504-1512.

Lactin, D. J., N. J. Holliday, D. L. Johnson and R. Craigen (1995). "Improved Rate Model of Temperature-Dependent Development by Arthropods." *Environmental Entomology* **24**(1): 68-75.

Lavola, A., R. Julkunen-Tiitto, H. Roininen and P. Aphalo (1998). "Host-plant preference of an insect herbivore mediated by UV-B and CO2 in relation to plant secondary metabolites." *Biochemical Systematics and Ecology* **26**(1): 1-12.

Li, C. Y., O. Junttila, P. Heino and E. T. Palva (2004). "Low temperature sensing in silver birch (Betula pendula Roth) ecotypes." *Plant Science* **167**(1): 165-171.

Liebhold, A., J. Elkinton, D. Williams and R. M. Muzika (2000). "What causes outbreaks of the gypsy moth in North America?" *Population Ecology* **42**(3): 257-266.

Lill, J. T. (2001). "Selection on herbivore life-history traits by the first and third trophic levels: The devil and the deep blue sea revisited." *Evolution* **55**(11): 2236-2247.

Lincoln, D. E. (1993). "The influence of plant carbon-dioxide and nutrient supply on susceptibility to insect herbivores." *Vegetatio* **104**: 273-280.

Lindroth, R. L., S. Roth, E. L. Kruger, J. C. Volin and P. A. Koss (1997). "CO2-mediated changes in aspen chemistry: Effects on gypsy moth performance and susceptibility to virus." *Global Change Biology* **3**(3): 279-289.

Logan, J. A., J. Regniere and J. A. Powell (2003). "Assessing the impacts of global warming on forest pest dynamics." *Frontiers in Ecology and the Environment* **1**(3): 130-137.

Logan, J. A., D. J. Wollkind, S. C. Hoyt and L. K. Tanigoshi (1976). "An analytical model for description of temperature dependent rate phenomena in arthropods." *Environmental Entomology* **5**: 1133-1140.

Lysyk, T. J. (1989). "Stochastic-model of eastern spruce budworm (Lepidoptera, Tortricidae) phenology on white spruce and balsam fir." *Journal of Economic Entomology* **82**(4): 1161-1168.

McCullough, D. G. (2000). "A review of factors affecting the population dynamics of jack pine budworm (Choristoneura pinus pinus Freeman)." *Population Ecology* **42**(3): 243-256.

Menzel, A. (2000). "Trends in phenological phases in Europe between 1951 and 1996." *International Journal of Biometeorology* **44**(2): 76-81.

Mols, C. M. M. and M. E. Visser (2002). "Great tits can reduce caterpillar damage in apple orchards." *Journal of Applied Ecology* **39**(6): 888-899.

Mopper, S. (2005). "Phenology - how time creates spatial structure in endophagous insect populations." *Annales Zoologici Fennici* **42**(4): 327-333.

Mopper, S., P. Stiling, K. Landau, D. Simberloff and P. Van Zandt (2000). "Spatiotemporal variation in leafminer population structure and adaptation to individual oak trees." *Ecology* **81**(6): 1577-1587.

Mousseau, T. A. and H. Dingle (1991). "Maternal effects in insect life histories." *Annual Review of Entomology* **36**: 511-534.

Mousseau, T. A. and C. W. Fox (1998). "The adaptive significance of maternal effects." *Trends in Ecology & Evolution* **13**(10): 403-407.

Murray, M. B., M. G. R. Cannell and R. I. Smith (1989). "Date of Budburst of Fifteen Tree Species in Britain Following Climatic Warming." *Journal of Applied Ecology* **26**(2): 693-700.

Musolin, D. L. and H. Numata (2003). "Timing of diapause induction and its life-history consequences in Nezara viridula: is it costly to expand the distribution range?" *Ecological Entomology* **28**(6): 694-703.

Nussey, D. H., E. Postma, P. Gienapp and M. E. Visser (2005). "Selection on heritable phenotypic plasticity in a wild bird population." *Science* **310**(5746): 304-306.

Nussey, D. H., A. J. Wilson and J. E. Brommer (2007). "The evolutionary ecology of individual phenotypic plasticity in wild populations." *Journal of Evolutionary Biology* **doi:10.1111/j.1420-9101.2007.01300.x**.

Ozaki, K. (1998). "Inter-specific difference in budburst time and its consequences on egg hatch time and survival of the gall-making adelgid *Adelges japonicus* (Monzen) (Hom., Adelgidae)." *Journal of Applied Entomology* **122**(8): 483-486.

Parmesan, C. and G. Yohe (2003). "A globally coherent fingerprint of climate change impacts across natural systems." *Nature* **421**(6918): 37-42.

Peterson, N. A. and A. C. Nilssen (1998). "Late autumn eclosion in the winter moth *Operophtera brumata*: compromise of selective forces in life-cycle timing." *Ecological Entomology* **23**(4): 417-426.

Pigliucci, M. (2001). *Phenotypic plasticity: beyond nature and nurture*. Baltimore, The John Hopkins University Press.

Porter, L. J., L. N. Hrstich and B. G. Chan (1985). "The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin." *Phytochemistry* **25**(1): 223-230.

Postma, E. and A. J. van Noordwijk (2005). "Genetic variation for clutch size in natural populations of birds from a reaction norm perspective." *Ecology* **86**(9): 2344-2357.

Powell, J. A. and J. A. Logan (2005). "Insect seasonality: circle map analysis of temperature-driven life cycles." *Theoretical Population Biology* **67**(3): 161-179.

Powell, L. E. (1987). The hormonal control of bud and seed dormancy in woody plants. *Plant hormones and their role in plant growth and development*. P. J. Davies. Dordrecht, Nijhoff**:** 539-552.

Pulido, F. and P. Berthold (2004). Microevolutionary response to climatic change. *Birds and Climate Change*. London, Academic Press Ltd. **35:** 151-183.

Quiring, D. T. (1992). "Rapid change in suitability of white spruce for a specialist herbivore, *Zeiraphera-Canadensis*, as a function of leaf age." *Canadian Journal of Zoology* **70**(11): 2132-2138.

Raymond, B., A. Vanbergen, A. Watt, S. E. Hartley, J. S. Cory and R. S. Hails (2002). "Escape from pupal predation as a potential cause of outbreaks of the winter moth, *Operophtera brumata*." *Oikos* **98**(2): 219-228.

Réale, D., A. G. McAdam, S. Boutin and D. Berteaux (2003). "Genetic and plastic responses of a northern mammal to climate change." *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**: 591-596.

Rinne, P., H. Hanninen, P. Kaikuranta, J. E. Jalonen and T. Repo (1997). "Freezing exposure releases bud dormancy in *Betula pubescens* and *B-pendula*." *Plant Cell and Environment* **20**(9): 1199-1204.

Roach, D. A. and R. D. Wulff (1987). "Maternal effects in plants." *Annual Review of Ecology and Systematics* **18**: 209-235.

Roland, J. (1994). "After the Decline - What maintains low winter moth density after successful biological control." *Journal of Animal Ecology* **63**(2): 392-398.

Roland, J. and D. G. Embree (1995). "Biological control of the winter moth." *Annual Review of Entomology* **40**: 475-492.

Root, T. L., J. T. Price, K. R. Hall, S. H. Schneider, C. Rosenzweig and J. A. Pounds (2003). "Fingerprints of global warming on wild animals and plants." *Nature* **421**(6918): 57-60.

Rossiter, M. C. (1991). "Environmentally-based maternal effects - a hidden force in insect population-dynamics." *Oecologia* **87**(2): 288-294.

Rossiter, M. C. (1991). "Maternal effects generate variation in life-history - Consequences of egg weight plasticity in the gypsy moth." *Functional Ecology* **5**(3): 386-393.

Rossiter, M. C. (1996). "Incidence and consequences of inherited environmental effects." *Annual Review of Ecology and Systematics* **27**: 451-476.

Rossiter, M. C., D. L. Coxfoster and M. A. Briggs (1993). "Initiation of maternal effects in *Lymantria Dispar* - Genetic and ecological components of egg provisioning." *Journal of Evolutionary Biology* **6**(4): 577-589.

Roy, D. B. and T. H. Sparks (2000). "Phenology of British butterflies and climate change." *Global Change Biology* **6**(4): 407-416.

Ruohomaki, K., S. Hanhimaki and E. Haukioja (1993). "Effects of egg size, laying order and larval density on performance of *Epirrita Autumnata* (Lep, Geometridae)." *Oikos* **68**(1): 61-66.

Ruohomaki, K., M. Tanhuanpaa, M. P. Ayres, P. Kaitaniemi, T. Tammaru and E. Haukioja (2000). "Causes of cyclicity of *Epirrita autumnata* (Lepidoptera, Geometridae): grandiose theory and tedious practice." *Population Ecology* **42**(3): 211-223.

Russell, F. L. and S. M. Louda (2004). "Phenological synchrony affects interaction strength of an exotic weevil with Platte thistle, a native host plant." *Oecologia* **139**(4): 525-534.

Sarvas, R. (1974). "Investigations on the annual cycle of development of forest trees II. Autumn dormancy and winter dormancy." *Communicationes Instituti Forestalis Fenniae* **84**: 1-101.

Schaber, J. and F. W. Badeck (2003). "Physiology-based phenology models for forest tree species in Germany." *International Journal of Biometeorology* **47**(4): 193-201.

Scheiner, S. M. (1993). "Genetics and evolution of phenotypic plasticity." *Annual Review of Ecology and Systematics* **24**: 35-68.

Schoolfield, R. M., P. J. H. Sharpe and C. E. Magnuson (1981). "Non-linear regression of biological temperature-dependent rate models based on absolute reaction rate theory." *Journal of Theoretical Biology* **88**: 719-731.

Selås, V., O. Hogstad, S. Kobro and T. Rafoss (2004). "Can sunspot activity and ultraviolet-B radiation explain cyclic outbreaks of forest moth pest species?" *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**(1551): 1897 - 1901.

Sharpe, P. J. H. and D. W. DeMichele (1977). "Reaction kinetics of poikilotherm development." *Journal of Theoretical Biology* **64**: 649-670.

Stenseth, N. C. and A. Mysterud (2002). "Climate, changing phenology, and other life history and traits: Nonlinearity and match-mismatch to the environment." *Proceedings of the National Academy of Sciences of the United States of America* **99**(21): 13379-13381.

Stenseth, N. C., A. Mysterud, G. Ottersen, J. W. Hurrell, K. S. Chan and M. Lima (2002). "Ecological effects of climate fluctuations." *Science* **297**(5585): 1292-1296.

Stevenson, D. E., G. Feng, R. J. Zhang and M. K. Harris (2005). "Physiological time model of Scirpophaga incertulas (Lepidoptera : Pyralidae) in rice in Guandong Province, People's Republic of China." *Journal of Economic Entomology* **98**(4): 1179-1186.

Stoyenoff, J. L., J. A. Witter and M. E. Montgomery (1994). "Gypsy moth (Lepidoptera, Lymantriidae) performance in relation to egg hatch and feeding initiation times." *Environmental Entomology* **23**(6): 1450-1458.

Strengbom, J., J. Witzell, A. Nordin and L. Ericson (2005). "Do multitrophic interactions override N fertilization effects on *Operophtera* larvae?" *Oecologia* **143**(2): 241-250.

Tammaru, T., M. Tanhuanpaa, K. Ruohomaki and A. Vanatoa (2001). "Autumnal moth - why autumnal?" *Ecological Entomology* **26**(6): 646-654.

Tanhuanpää, M., K. Ruohomaki, P. Kaitaniemi and T. Klemola (1999). "Different impact of pupal predation on populations of *Epirrita autumnata* (Lepidoptera; Geometridae) within and outside the outbreak range." *Journal of Animal Ecology* **68**(3): 562-570.

Tanhuanpaa, M., K. Ruohomaki and E. Uusipaikka (2001). "High larval predation rate in non-outbreaking populations of a geometrid moth." *Ecology* **82**(1): 281-289.

Tauber, C. A. and M. J. Tauber (1981). "Insect Seasonal Cycles: Genetics and Evolution." *Annual Review of Ecology and Systematics* **12**: 281-308.

Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. F. de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley,

A. S. van Jaarsveld, G. F. Midgley, L. Miles, M. A. Ortega-Huerta, A. T. Peterson, O. L. Phillips and S. E. Williams (2004). "Extinction risk from climate change." *Nature* **427**(6970): 145-148.

Thomson, A. J., R. F. Shepherd, J. W. E. Harris and R. H. Silversides (1984). "Relating weather to outbreaks of western spruce budworm, *Choristoneura Occidentalis* (Lepidoptera, Tortricidae), in British-Columbia." *Canadian Entomologist* **116**(3): 375-381.

Tikkanen, O. P. and R. Julkunen-Tiitto (2003). "Phenological variation as protection against defoliating insects: the case of *Quercus robur* and *Operophtera brumata*." *Oecologia* **136**(2): 244-251.

Tikkanen, O. P. and P. Lyytikainen-Saarenmaa (2002). "Adaptation of a generalist moth, *Operophtera brumata*, to variable budburst phenology of host plants." *Entomologia Experimentalis Et Applicata* **103**(2): 123-133.

Tikkanen, O. P., P. Niemela and J. Keranen (2000). "Growth and development of a generalist insect herbivore, *Operophtera brumata*, on original and alternative host plants." *Oecologia* **122**(4): 529-536.

Topp, W. and K. Kirsten (1991). "Synchronization of preimaginal development and reproductive success in the winter moth, *Operophtera Brumata* L." *Journal of Applied Entomology* **111**(2): 137-146.

Turchin, P. (1999). "Population regulation: a synthetic view." *Oikos* **84**(1): 153-159.

Van Asch, M., P. H. Van Tienderen, L. J. M. Holleman and M. E. Visser (2006). "Predicting adaptation to climate change, an insect herbivore example." *Global Change Biology* **In press**.

Van Asch, M. and M. E. Visser (2007). "Phenology of forest caterpillars and their host trees: the importance of synchrony." *Annual Review of Entomology* **52**: 37-55.

Van Dongen, S., T. Backeljau, E. Matthysen and A. A. Dhondt (1997). "Synchronization of hatching date with budburst of individual host trees (*Quercus robur*) in the winter moth (*Operophtera brumata*) and its fitness consequences." *Journal of Animal Ecology* **66**(1): 113-121.

Van Dongen, S., E. Matthysen and A. A. Dhondt (1996). "Restricted male winter moth (*Operophtera brumata* L) dispersal among host trees." *Acta Oecologica* **17**(4): 319-329.

Van Tienderen, P. H. and H. P. Koelewijn (1994). "Selection on reaction norms, genetic correlations and constraints." *Genetical Research* **64**(2): 115-125.

Van Zandt, P. A. and S. Mopper (1998). "A meta-analysis of adaptive deme formation in phytophagous insect populations." *American Naturalist* **152**(4): 595-604.

Varley, G. C. and G. R. Gradwell (1960). "Key factors in population studies." *Journal of Animal Ecology* **29**(2): 399-401.

Varley, G. C. and G. R. Gradwell (1968). Population models for the winter moth. *Insect abundance*. S. T. R. E. Oxford, Blackwell Scientific**:** 132-142.

Verbeek, K. (2003). De toestand van het klimaat in Nederland 2003. De Bilt, Royal Dutch Metereological Institute (KNMI)**:** 1-32.

Visser, M. E. and C. Both (2005). "Shifts in phenology due to global climate change: the need for a yardstick." *Proceedings of the Royal Society of London B-Biological Sciences* **272**(1581): 2561-2569.

Visser, M. E., C. Both and M. M. Lambrechts (2004). Global climate change leads to mistimed avian reproduction. *Birds and Climate Change*. London, Academic Press Ltd. **35:** 89-110.

Visser, M. E. and L. J. M. Holleman (2001). "Warmer springs disrupt the synchrony of oak and winter moth phenology." *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**: 289-294.

Visser, M. E., A. J. van Noordwijk, J. M. Tinbergen and C. M. Lessells (1998). "Warmer springs lead to mistimed reproduction in great tits (*Parus major*)." *Proceedings of the Royal Society of London Series B-Biological Sciences* **265**: 1867-1870.

Wagner, T. L., R. L. Olson and J. L. Willers (1991). "Modeling arthropod development time." *Journal of Agricultural Entomology* **8**(4): 251-270.

Wagner, T. L., H. I. Wu, P. J. H. Sharpe, R. M. Schoolfield and R. N. Coulson (1984). "Modeling insect development rates - a literature-review and application of a biophysical model." *Annals of the Entomological Society of America* **77**(2): 208-225.

Watt, A. D. and A. M. McFarlane (1991). "Winter moth on Sitka spruce - Synchrony of egg hatch and budburst, and its effect on larval survival." *Ecological Entomology* **16**(3): 387-390.

Watt, A. D. and I. P. Woiwod (1999). "The effect of phenological asynchrony on population dynamics: analysis of fluctuations of British macrolepidoptera." *Oikos* **87**(2): 411-416.

Wijngaarden, P. J. and P. M. Brakefield (2001). "Lack of response to artificial selection on the slope of reaction norms for seasonal polyphenism in the butterfly *Bicyclus anynana*." *Heredity* **87**(4): 410-420.

Wijngaarden, P. J., P. B. Koch and P. M. Brakefield (2002). "Artificial selection on the shape of reaction norms for eyespot size in the butterfly *Bicyclus anynana*: direct and correlated responses." *Journal of Evolutionary Biology* **15**(2): 290-300.

Wint, G. R. W. (1983). *The effect of foliar nutrients upon the growth and feeding of a Lepidopteran larva*. Nitrogen as an ecological factor; the 22nd symposium of the British ecological society, Oxford, 1981, Oxford, Blackwell.

Wint, G. R. W. (1983). "The role of alternative host-plant species in the life of a polyphagous moth, *Operophtera brumata* (Lepidoptera: Geometridae)." *Journal of Animal Ecology* **52**(2): 439-450.

Wint, W. (1983). "The role of alternative host-plant species in the life of a polyphagous moth, *Operophtera brumata* (Lepidoptera, Geometridae)." *Journal of Animal Ecology* **52**(2): 439-450.

Wolda, H. (1988). "Insect seasonality: why?" *Annual Review of Ecology and Systematics* **19**: 1-18.

Woltereck, R. (1909). "Weiterer experimentelle Untersuchungen uber Artveranderung, Speziell uber das Wessen Quantitativer Artunterschiede bei Daphniden." *Verh. Deutsch. Zool. Ges.* **19**: 110-172.

Zalucki, M. P., A. R. Clarke and S. B. Malcolm (2002). "Ecology and behavior of first instar larval Lepidoptera." *Annual Review of Entomology* **47**: 361-393.

Nederlandse samenvatting

In elk ecosysteem zijn interacties tussen soorten van het grootste belang. In een gematigd klimaat, zoals dat in Nederland voorkomt, is het voorkomen van veel plant- en diersoorten sterk seizoensgebonden. Vaak is er maar een zeer beperkte periode in het jaar geschikt voor groei en/of reproductie. Wanneer deze periode valt, hangt af van zowel abiotische en biotische factoren. Een belangrijke abiotische factor is temperatuur. Voorbeelden van biotische factoren zijn bijvoorbeeld aan- of afwezigheid van voedselplanten of predators. Niet alleen is vaak een beperkte periode geschikt voor groei, de optimale periode kan ook sterk verschillen van jaar tot jaar; er is sprake van temporele variatie. Hoe organismen hiermee om gaan, zodanig dat ze toch elk jaar op het goede moment aanwezig zijn, is het onderwerp van dit proefschrift. Een belangrijke vraag is ook wat voor gevolgen klimaatsverandering heeft. Door klimaatsverandering zal de optimale periode veranderen. Hoe zal dit een soort beïnvloeden? Kan een organisme zich aanpassen aan de veranderde omstandigheden? En hoe snel kan een soort zich dan aanpassen?

Tijdens mijn onderzoek heb ik de kleine wintervlinder (*Operophtera brumata*) levend op eik (*Quercus robur*) als modelsysteem gebruikt. Wintervlinders hebben een éénjarige levenscyclus: het vrouwtje legt de eieren in het najaar in de boom, en deze eieren komen het volgende voorjaar uit. De rupsen zijn afhankelijk van jong eikenblad; ouder blad wordt al snel oneetbaar. Omdat het blad maar gedurende een zeer korte periode geschikt is als voedsel, is het moment van ei-uitkomst ten opzichte van het uitlopen van de eikenbomen van groot belang voor de wintervlinder. Bovendien varieert het moment dat de bomen uitlopen sterk tussen jaren en tussen bomen. Timing van ei-uitkomst met het uitlopen van de bomen verschilt ook sterk tussen de jaren. Met name in warme jaren komen de eieren uit vóórdat de bomen uitlopen.

Voordat het mogelijk is om iets over timing tussen vlinder en boom te zeggen, moeten we eerst begrijpen welke factoren ei-uitkomst beïnvloeden (hoofdstuk 3). Ei-uitkomst wordt door de temperatuur bepaald: bij hoge temperatuur komen de eieren eerder uit. Ei-uitkomst wordt niet beïnvloed door daglengte. Wel neemt de temperatuurgevoeligheid toe tijdens de ontwikkeling. De ontwikkeling begint wel meteen nadat de eieren gelegd zijn, er is geen (volledige) rustfase. Dit is waarschijnlijk de verklaring voor de mistiming tussen eik en wintervlinder in recente warme jaren: de eieren van de wintervlinder ontwikkelen sneller door de hogere wintertemperatuur, terwijl de bomen op dat moment nog in rust zijn en dus veel minder sterk worden beïnvloed door de temperatuur in de winterperiode.

Een niet genetisch effect dat ei-uitkomst kan beïnvloeden, is voedselkwaliteit (timing) van de ouders (hoofdstuk 4). Als een vrouwtje relatief laat uit het ei komt, eet ze ouder blad, en gaat daardoor eerder verpoppen. Hierdoor legt ze eerder haar eieren, en dus komen deze eieren relatief ook eerder uit. Dit kan echter geen oplossing zijn voor de effecten van de klimaatsverandering, omdat dit leidt tot uitkomen van de eieren vóórdat er blad

beschikbaar is voor de rupsen. Dit overleven de rupsen niet, en dus kan dit maternale effect hierbij geen rol spelen.

De wintervlinder reageert te sterk op de temperatuur. Om zich aan te kunnen passen aan de veranderde omstandigheden, moet de temperatuur respons van de eieren veranderen. Aanpassing kan plaatsvinden als er *a*) genoeg variatie in het kenmerk is, *b*) deze variatie genetisch bepaalt is, d.w.z. de nakomelingen lijken op de ouders, en *c*) er voldoende selectie is. Er is genetische variatie in ei-uitkomst, en gekoppeld aan de sterke selectie, voorspellen we een snelle verandering te zien, die leidt tot een betere synchronisatie met de eik (hoofdstuk 5). In hoofstuk zes laat ik tenslotte zien dat de temperatuurrespons van de wintervlindereieren de afgelopen tien jaar veranderd is. Bij dezelfde temperatuur komen de eieren nu vijf tot tien dagen later uit dan tien jaar geleden. Aangezien de temperatuur sterk verschilt tussen jaren, kan toch de ei-uitkomst tussen jaren ook nog wel sterk verschillen. Deze verandering komt overeen met de voorspelde verandering (hoofdstuk 5).

Dankwoord

Eindelijk is het dan zover, na heel wat ploeteren en zwoegen is dit boekje klaar. Veel mensen zijn in de loop van de tijd direct of indirect betrokken geweest bij het tot stand komen ervan. Niet alleen op wetenschappelijk gebied, maar zeker ook daarbuiten, hebben alle collega's, vrienden en familie een grote rol gespeeld in de afgelopen vier jaar. Mijn periode op het NIOO heb ik dan ook niet alleen als zeer nuttig en leerzaam, maar bovenal ook als enorm leuke en gezellige tijd ervaren. Graag wil ik hierbij dan ook deze mensen bedanken.

Allereerst wil ik graag mijn begeleider en promotor bedanken. Marcel heeft een grote rol gespeeld bij het bepalen van het onderzoek, maar ook gaf hij me altijd de vrijheid en stimuleerde hij me om mijn eigen ideeën te ontwikkelen. Hierdoor heb ik veel geleerd over zelfstandig je eigen onderzoek ontwikkelen, en hiervoor ben ik zeer dankbaar. De vele discussies over experimenten en de resultaten daarvan hebben dit proefschrift enorm verbeterd.

Ook wil ik bij deze Leonard bedanken. Hij introduceerde de rupsen en studiegebieden aan me, en leerde me de (on)mogelijkheden van het praktisch werk. Verder stond hij altijd klaar met hulp en advies bij het uitvoeren van de experimenten. Ik heb dankbaar gebruik gemaakt van alle ervaring die hij had opgedaan voordat ik aan dit project begon, en ook bij het verzamelen van de lange-termijngegevens heeft hij een cruciale rol gespeeld.

Kate leerde me om heel kritisch naar mijn eigen proefopzetten en resultaten te kijken, en heeft zowel inhoudelijk als grammaticaal mijn manuscripten sterk verbeterd. Piet en Arie hebben tijdens werkbesprekingen en discussies waardevolle suggesties gedaan. Erik, Eva, Phillip, Kees, Toby, Christiaan, Arnold en Fransisco zorgden voor zowel voor wetenschappelijke input tijdens de Science-lunch en Journal Club, en ook voor een hoop gezelligheid in de werkgroep. Piet leerde me 'Warnsborn bij nacht' kennen, Christa en Agaat stonden altijd klaar als ik weer eens hun vooraad kwam plunderen, en Rinse zorgde voor de nodige mobiliteit. Henk leerde me met de foto-en meetapparatuur omgaan en Wieche hielp me met de kleurreacties om tannines in mijn eikenbladeren te bepalen. Ook de OIO's van de andere werkgroepen zorgden voor veel gezelligheid, met name Joanneke, Paul, Annemieke, Annelies en Jelmer wil ik van harte daarvoor bedanken!

In de afgelopen periode zijn ook meerdere studenten betrokken geweest bij het wintervlinderwerk. Rick, Jildou, Kim, Stefan en Jan hebben met veel enthousiame meegewerkt binnen hun eigen projecten. Ik hoop dat jullie het net zo leuk hebben gevonden, en er net zoveel van geleerd hebben als ik. Tanya sacrified her time off in order to help me get started with one of the largest experiments. Lisa nam al het standaardwerk over toen mijn project stopte, en heeft bovendien nog volledig zelfstandig een aanvullend experiment gedaan wat nog belangrijke informatie oplevert in aanvulling op mijn gegevens.

Buiten het NIOO heeft Peter van Tienderen een grote rol gespeeld bij het tot stand komen van hoofdstuk 5, en mij bovendien met veel geduld leren rekenen aan

reactienormen. Many thanks also to Riitta Julkunen-Tiito, who taught me a lot about HPLC, and who kindly allowed me to perform analyses on my leaves in her lab in Joensuu. Mijn allereerste ervaringen met onderzoek doen en met populatiebiologie was bij Gerdien. Tijdens mijn OIO-periode leerde ze me de basis van de physiologische modellen zoals ik die gebruik in hofdstuk 3. Ze heeft dan ook een belangrijke bijdrage geleverd aan het tot stand komen van dit hoofdstuk.

Tevens gaat ook mijn dank uit naar de terreineigenaren en –beheerders. Barones Van Boetzelaer Van Oosterhout, Gemeente Renkum, Nationaal Park De Hoge Veluwe, Staatsbosbeheer, Stichting het Gelders Landschap, Stichting het Utrechts Landschap en Vereniging Natuurmonumenten stemden erin toe dat ik in hun terreinen vallen ophing, en maakten dit onderzoek daarmee mogelijk.

Tenslotte wil ik ook graag mijn familie bedanken. Steeds als het weer eens niet wilde, of iets toch ietsje meer tijd kostte, waren jullie er om me op te vangen en op te beuren. Zonder jullie steun was het niet gelukt om dit boekje te maken!

Curriculum Vitae

I was born in 1979, and after finishing high school in Culemborg I went to Utrecht University in 1997. My first project there was at the Evolutionary Population Biology group. I focused in this project on phenotypic and genetic differences in body size between geographically different populations of *Drosophila melanogaster*, and looked at the trade-off between different fitness components in these populations. I did my second Master-project at the Behavioral Biology Department. Here, my project was part of a larger project, dealing with the genetic basis of aggressive behaviour in Golden Retrievers. Within this project I looked especially at the predictive value of threatening behaviour, using standard testing facilities. Finally, I did a literature survey on the existence of wing-dimorphism.

After finishing Biology in Utrecht, I started as a PhD-student at the NIOO in Heteren in 2002. I am interested in the interaction between different trophic levels, and during my PhD I focused on timing of herbivore appearance in relation to the host plant, using the winter moth (*Operophtera brumata*) egg hatch – oak (*Quercus robur*) bud opening as a study system. The results of this work are presented in this thesis.

Currently I am working as a Post-Doc at the University of Oxford. Here I will look at genetic variation in aphids and in their secondary endosymbionts, and in particular I will focus on various fitness effects of different genetic combinations and the effects on the population dynamics of the aphid system under various conditions.

Publications

Publications

Van Asch, M. and ME Visser (2007). Phenology of forest caterpillars and their host trees: the importance of synchrony. *Annual Review of Entomology* **52**: 37-55.
Chapter 2

Van Asch M, van Tienderen PH, Holleman LJM and Visser ME. Predicting adaptation of phenology in response to climate change, an insect herbivore example. *Global Change Biology*. *In press.*
Chapter 5

Submitted & in preparation

Van Asch M, G de Jong and ME Visser. Modeling *Operophtera brumata* egg phenology: nonlinear effects of temperature and of developmental stage on development.
Chapter 3

Van Asch M, R Julkunen-Tiito and ME Visser. Maternal effects in an insect herbivore as a mechanism to adapt to host plant phenology.
Chapter 4

Van Asch M, LJH Holleman and ME Visser. Climate change induced genetic shifts in an insect herbivore's temperature sensitivity.
Chapter 6

Both C, M van Asch, RG Bijlsma and ME Visser. Phenological changes across four trophic levels: oaks, caterpillars, passerines and sparrowhawks.

Addresses of co-authors

Leonard H. Holleman	Netherlands Institute of Ecology (NIOO-KNAW) Centre for Terrestrial Ecology P.O. Box 40 6666 ZG Heteren, The Netherlands
Gerdien de Jong	Utrecht University Department of Biology Padualaan 8 3584 CH Utrecht, The Netherlands
Riitta Julkunen-Tiitto	University of Joensuu Department of Biology Yliopistokatu 7 FIN-80110 Joensuu, Finland
Peter H. van Tienderen	University of Amsterdam Institute for Biodiversity and Ecosystem Dynamics Kruislaan 318 1098 SM Amsterdam, The Netherlands
Marcel E. Visser	Netherlands Institute of Ecology (NIOO-KNAW) Centre for Terrestrial Ecology P.O. Box 40 6666 ZG Heteren, The Netherlands